Land-use responses of dung beetle communities and their ecosystem services

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Eco Networks

Land-use responses of dung beetle communities and their ecosystem services

Für meine Familie.

“Knowing is not enough, we must apply –

Willing is not enough, we must do.”

Bruce Lee

Contents

Summary

Global urbanization and rising human population densities result in a constant need for resources and food. Land use and land cover are inevitably bound to human population dynamics and thus remain the major drivers of continuous environmental change. Agricultural land use and forestry affect species communities and consequently their ecological functions – such as nutrient cycling or protection against soil erosion – resulting in a loss of ecosystem services. Indicator species, therefore, provide valuable contributions for the observation of anthropogenic disturbance, as they respond sensitively towards changes of their habitat and living conditions. The presence or absence of such species affects the evaluation of disturbances and predictions of ecosystem changes and thus might reveal functional consequences.

Detritivores (Saprobionts) utilize and process organic material, which otherwise would be inaccessible for higher trophic levels. Their unique way of recycling organic material renders them important contributors on the base of every food web. Hence, in context of agricultural land use and forestry in differing management intensities, I focused within this thesis on the occurrence, ecosystem services, community structure and behavioral patterns of a basal superfamily of insects: dung beetles (Coleoptera: Scarabaeoidea). Dung beetles are widespread in most habitats around the globe and represent important ancient and current detritivores. These functionally important beetles are faced with a wide range of anthropogenic disturbances and changes in environmental conditions due to land use. I thus conducted quantitative surveys of the abundance of dung beetles and their dung removal rates in forest and grassland sites with varying land-use intensity, to focus on following research questions: (Q1) *Does land use affect dung beetles and their ecosystem services?* (Q2) *In which ways do dung beetle-resource connectivity and the complexity of this trophic network respond to (rising) land-use intensity?* (Q3) *Do dung beetle – resource interactions change in specificity along a global, latitudinal gradient?*

Besides these applied and ecosystem-service related questions I found patterns and results for the beetles' resource preference, which were discussed controversially in the literature and still remain to be fully understood. For a better and more basic understanding of this detritivorous group of insects I addressed the following questions: (Q4) *Is the nutritional value of dung a driving force for dung type attractiveness and dung beetle preference?* (Q5) *Which roles have volatile organic compounds in dung beetle attraction?*

In chapter 2 I used dung from livestock and game animals to provide a characteristic spectrum of dung resources and sampled 300 experimental sites, including forests and grasslands. Since every sampling site differed in management intensity, I was able to calculate the effects of rising land-use and forest management and highlight contrary, but foremost negative effects on dung removal for both habitats. Chapter 3 is a more indepth analysis of the beetles' community structure and the complexity of dung beetle networks. The species' abundance and distribution across sites revealed a generalization of beetle-resource interactions, which led to more even and higher decomposition rate. Additionally, I found that a rising dung beetle network complexity translates into an enhanced robustness against land use. To test the beetles' resource specificity on a global scale, I conducted in chapter 4 a meta-analysis of 110 dung beetle-resource interaction networks along a latitudinal gradient. Despite a significant increase of dung beetle diversity towards the equator, overall the dung beetle networks remained highly generalistic. In chapter 5 I conducted nutritional analyses (amino acids, fatty acids, sterols, C/N contents) of different dung types to unravel patterns in dung type preferences I observed in the field. Albeit differences in nutritional composition on a feeding guild level, these results did not predict patterns of dung preference. Subsequently, I analyzed volatile organic compounds of different dung types in chapter 6. Dung scent components (as described in the literature or elucidated by my own gas chromatographic measurements) were used in single and mixed baits to test for attractivity, compared to natural dung samples. Dung scent analyses revealed both, unique bouquets and ubiquitous volatiles for different dung types. This leads to specific volatile blends – including key volatiles – for the beetles' resource localization.

In summary, this thesis contributes to applied issues regarding land use and forestry, conveys an enhanced understanding of the (global) community structure and faces issues in basic dung-beetle research. Anthropogenic disturbance, like habitat dependent management, often negatively affects ecosystem services of various taxa; also true for some responses of

dung beetles i.e. the beetles' dung removal in terms of deforestation. Yet, dung beetle communities show an unexpected robustness against land-use intensity. Due to the high generalization level of dung beetle networks, so far, a balanced dung removal is assured. Even on a global basis, this generalistic character of dung beetle-resource interactions remains similar across a latitudinal gradient. Observed resource specificity cannot be explained by (differing) nutritional values of the dung types, but certain mixtures and single (key) volatile organic compounds seem crucial for specific patterns in dung beetle attraction.

Zusammenfassung

Die globale Besiedlung und steigende Bevölkerungsdichten verursachen einen konstanten Bedarf an Ressourcen und Nahrung. Eine großflächige Bebauung und Landnutzung sind daher unausweichlich mit menschlichen Populationsdynamiken verbunden und stellen eine der größten Triebkräfte für konstante Veränderungen in unserer Umwelt dar. Landwirtschaftliche Bodennutzung und Forstwirtschaft beeinflussen Artengemeinschaften und deren Ökosystemfunktion – wie beispielsweise die Unterstützung von Nährstoffkreisläufen oder Schutz vor Bodenerosion – was schlussendlich zu einem Verlust von Ökosystemdienstleistungen führen kann. Indikator-Spezies sind ein nützliches Hilfsmittel zur Evaluierung von anthropogen versursachten Störungen, da diese meist sehr sensibel auf Veränderungen in ihrem Habitat reagieren. Die An- oder Abwesenheit solcher Arten hilft bei der Erstellung von Modellen, die das Ausmaß von Störungen erheben und dadurch Prognosen möglicher Auswirkungen für unser Ökosystem ermöglichen.

Detritivore (Saprobionten) verwenden und prozessieren organisches Material, das andernfalls für höhere trophische Ebenen kaum zugänglich wäre. Durch ihre einzigartige Nutzung und Verwertung von solch organischem Material leisten sie einen extrem wichtigen Beitrag auf den untersten Stufen der Nahrungsnetze. Aus diesem Grund habe ich mich in meiner Dissertation mit dem Vorkommen, den Ökosystemdienstleistungen, Strukturen in der Artengemeinschaft und Verhaltensmustern einer basalen Großfamilie von Insekten im Kontext von Land- und Forstwirtschaft mit unterschiedlichen Management-Intensitäten gewidmet: den Dungkäfern (Coleoptera: Scarabaeoidea). Dungkäfer sind weltweit in den meisten Habitaten verbreitet und repräsentieren seit dem Jura eine extrem wichtige Gruppe von Detritivoren. Durch Landnutzung sind sie einem breiten Spektrum von anthropogenen Störungen und Veränderungen in ihren Lebensräumen ausgesetzt. Daher habe ich in quantitativen Untersuchungen die Abundanz der Käfer sowie die Dung-Abbauraten im Wald und Grünland bei unterschiedlich starker Land- und Forstwirtschaft durchgeführt, um

folgende Fragen zu beantworten: (Q1) Werden *Dungkäfer und ihre Funktion für das Ökosystem durch Land- und Forstwirtschaft beeinflusst?* (Q2) *Wie wirkt sich die (steigende) Bewirtschaftung von Wäldern und Wiesen auf die Nutzung der (Dungkäfer-) Ressourcen und die daraus resultierende Komplexität dieser trophischen Netzwerke aus?* (Q3) *Ändert sich die Komplexität und damit die Spezialisierung der Netzwerke „Dungkäfer – Ressource" entlang der Breitengrade?*

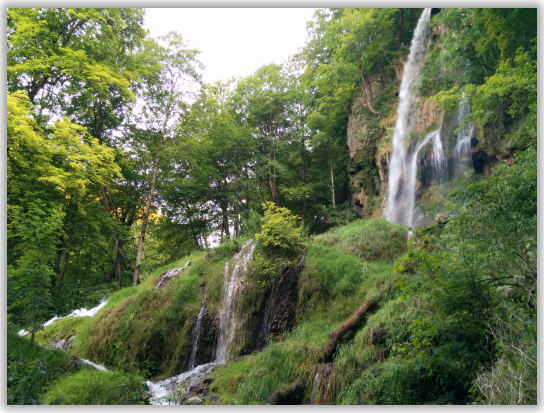
Zusätzlich zu diesen eher angewandten Fragestellungen habe ich während meiner Arbeit mit den Dungkäfern Muster in der Präferenz für bestimmte Ressourcen gefunden. Gründe hierfür werden in der Literatur noch immer kontrovers diskutiert und sind noch nicht ausreichend geklärt. Daher habe ich für ein besseres und grundlegenderes Verständnis dieser detritivoren Gruppe zusätzlich folgende Fragen bearbeitet: (Q4) *Ist der Nährstoffgehalt ausschlaggebend für die Attraktivität und die Auswahl bestimmter Dungsorten?* (Q5) *Welche Rolle spielen volatile, organische Komponenten bei der Attraktivität von Dung und die Auswahl zwischen verschiedenen Ressourcen der Dungkäfer?*

In Kapitel 2 beschreibe ich die Beprobung von 300 Experimentalflächen in Wäldern und Wiesen, wobei verschiedene Dungsorten von Nutz- und Wildtieren verwendet wurden, um ein möglichst natürliches Ressourcenspektrum für Dungkäfer abzudecken. Da die einzelnen Experimentalflächen in der Intensität der Bewirtschaftung variiert haben, konnte ich den Einfluss von steigender Land- und Forstwirtschaft auf den Dungabbau in beiden Habitaten untersuchen, wobei sich teils gegenläufige aber primär negative Effekte herausgestellt haben. Kapitel 3 ist eine tiefergehende Analyse der beprobten Dungkäfergemeinschaften und deren Komplexität. Die Abundanz und Verteilung der Arten über die Versuchsflächen zeigt ein generalistisches Dungkäfer-Ressource Verhältnis, welches zu einem erhöhten und gleichmäßigerem Dungabbau führt. Zusätzlich konnte ich zeigen, dass eine erhöhte Komplexität der Dungkäfer-Ressourcen Interaktion zu einer erhöhten Resistenz gegenüber Bewirtschaftung führt. Um eine Ressourcenpräferenz der Dungkäfer auf globaler Ebene zu untersuchen, habe ich in Kapitel 4 eine Meta-Analyse mit 110 verschiedenen Interaktionsnetzwerken zwischen Dungkäfer und ihren Ressourcen entlang eines Breitengrad-Gradienten durchgeführt. Die Diversität der Dungkäfergemeinschaften nimmt zwar in Richtung des Äquators zu, die Interaktionsnetzwerke (und damit der Spezialisierungsgrad der Tiere) bleibt jedoch hoch generalistisch. In Kapitel 5 habe ich eine Nährstoffanalyse (Aminosäuren, Fettsäuren, Sterole, C/N-Verhältnis) verschiedener Dungsorten durchgeführt, um die Ursache von Dungpräferenzen zu untersuchen, die ich während der Feldversuche

dokumentieren konnte. Obwohl sich die Dungsorten in ihrer Nährstoffzusammensetzung auf einem Fraßgilden-Niveau unterscheiden (Carnivore, Omnivore und Herbivore Dungproduzenten), können die Ergebnisse die Dungpräferenzen nicht vollständig erklären. Daher habe ich in Kapitel 6 verschiedene Duftkomponenten der Dungsorten verwendet (auf Literaturbasis und aus eigenen gas-chromatographischen Messungen identifiziert), um deren Attraktivität als Einzel– oder Mischköder im Vergleich zu natürlichem Dung zu testen. Eine zusätzliche Duftstoffanalyse der im Feld verwendeten Dungsorten zeigt sowohl einzigartige Bouquets als auch ubiquitäre Duftkomponenten in den einzelnen Dungsorten. Daraus lässt sich schließen, dass die Dungkäfer sowohl spezifische Duftgemische, als auch bestimmte Schlüsselkomponenten zur Suche und Auswahl ihrer Ressourcen verwenden.

Die vorliegende Dissertation trägt zu einem besseren Verständnis der Auswirkungen von Land- und Forstwirtschaft bei, vermittelt tiefere Erkenntnisse über die (globalen) Strukturen der Artengemeinschaften und behandelt grundlegende Fragestellungen in Bezug auf Dungkäfer. Anthropogene Störungen wie Landnutzung haben häufig negative Auswirkungen auf die Funktion verschiedener Taxa im Ökosystem, was hier beispielsweise durch eine Beeinträchtigung des Dungabbaus durch Holzeinschlag auch für Dungkäfer bestätigt werden kann. Darüber hinaus zeigen jedoch die untersuchten Dungkäfergemeinschaften eine unerwartet hohe Toleranz gegenüber (steigender) Landnutzung. Aufgrund der generalistisch ausgeprägten Interaktionsnetzwerke kann vorerst ein gleichmäßiger Dungabbau seitens der Käfer gewährleistet werden. Der generalistische Charakter dieser Tiergruppe in Bezug auf ihre Ressourcenwahl bleibt ebenfalls global über die verschiedenen Breitengrade hinweg erhalten. Beobachtete Muster in der Auswahl und Präferierung der verschiedenen Dungsorten können zwar nicht durch eine unterschiedliche Nährstoffzusammensetzung erklärt werden, allerdings gibt eine nähere Analyse der Dunftkomponenten im Dung Hinweise auf die Nutzung bestimmter Bouquets und einzelner Volatile als Hauptfaktor oder mögliche Auslöser für eine gerichtete Ressourcenwahl bei Dungkäfern.

Chapter 1



1 General Introduction

Since first settlements and urban development, the environment has faced constant changes due to human activity (Vitousek et al. 1986 Bioscience 1997 Science). The continuous but likewise inevitably use and transformation of ecosystems is a major driver for long lasting changes in landscapes and impacts on the surrounding areas (Sala et al. 2000, Lambin et al. 2003). Quite frequently, direct and indirect effects of anthropogenic interference are delayed and the prediction of consequences is severe (McKee et al. 2004, Dawson et al. 2011). The mandatory need for cultivation- and living areas requires strategies for efficient, but especially sustainable land use and urban development, because uncontrolled land degradation will lead to substantial costs (Daily 1995 Science). Fortunately, a proceeding awareness and changed view on interaction between humans and their environment is increasingly focused, and still puzzling science (Tilman et al. 2017). Thus, the evaluation of recent and upcoming conflicts between nature conservation and human land use is an exceedingly challenging task for ecologists.

Agriculture and forestry represents types of management, which massively induce habitat conversion and by this affect local biodiversity and species communities (Newbold et al. 2015). As biodiversity, driven by varying species and communities, is linked to ecosystem services and vice versa, ecological functions within forests and grasslands face disturbance and alteration, consequently leading to a loss of ecosystem services (Cardinale et al 2012, Naeem et al. 1994) (Krebs et al. 1999, Tilman et al. 2001). This state of ubiquitous linkage and mandatory connections highlights the importance of local-scale studies for suitable characterization and projection of land use on (local) biodiversity and ecosystem services (Alan et al. 2015, Soliveres et al. 2016). Indicator species, therefore, are a valuable “tools” to observe anthropogenic disturbance, as they react sensitively to changes of their habitat and living conditions. Thus, the presence or absence of such species supplies models for functional consequences and facilitates the evaluation of disturbances and predictions of ecosystem changes (Kremen et al. 1993, Brown 1997).

1.1 **Biodiversity Exploratories and quantification of management**

To approach such wide-ranging issues, it is helpful to break down the high complexity of multi-dimensional interactions within an ecosystem and focus on smaller parts, such as specific groups of organisms. In fact, this still is a broad point of view, but it provides a framework within a more feasible context. The German Research Foundation, therefore, initiated a national infrastructure project platform: the exploratories for large-scale and long-term functional biodiversity research (Biodiversity Exploratories). Within this framework, the overarching aim is "the understanding of the relationship between biodiversity of different taxa and levels, the role of land use and management for biodiversity and the role of biodiversity for ecosystem processes" (www.biodiversity-exploratories.de). The Biodiversity Exploratories were established within three regions across Germany (Fig.1.1), including 100 randomly chosen experimental plots (per region) in forests and grasslands (50 plots each):

Figure 1.1: Location of the Biodiversity Exploratories. Source: Universität Münster

The Biosphere Reserve Schorfheide-Chorin in the northeast of Germany represents glacially formed lowland with large areas of pine and beech forests. Although the Schorfheide is one of the driest areas in Germany (annual precipitation: about 520 – 580 mm), lakes and moors are very common in this region. Most farms cultivate more than 500 ha of land, which demonstrates the agricultural value of the surrounding areas of the Schorfheide.

The Hainich-Dün region in the center of Germany contains with its national park of unmanaged mixed beech forest one of the largest closed forest areas in Germany (16000 ha). In contrast, the surrounding areas are characterized by fertile soils and thus by agricultural land.

The Schwäbische Alb, in southwest Germany, represents a mosaic of forests (old-growth beech, mixed forests and spruce monocultures) and grasslands (heathland, meadows and agricultural land) in the center of a calcareous mountain range (460 – 860 m a.s.l.).

In general, agriculture and forestry differs in structure and intensity, depending on management systems (organic versus integrative management), aims of the stakeholders and regional conditions (e.g. plains versus heaths) (Herzog et al. 2006). Yet, besides a broad

variety of single effective elements, the selection of main factors of habitat management allows a quantification of anthropogenic impact: while grazing, fertilization and mowing are crucial for grassland management (hereafter called land use) (Blüthgen et al. 2012), timber harvest, the plantation non-native tree species (i.e. pine and spruce) and the occurrence of deadwood are of central importance within forests (Kahl and Bauhus 2014). Consequently, these key components of land use and forest management – applied uniformly within a large study area – facilitate the quantification of anthropogenic disturbance. In total, the three regions of the Biodiversity Exploratories, including the experimental plots, cover about 10 % of agricultural land, pastures and forests of Germany. As the experimental plots within each region cover a representative range of forest management and land-use intensity, the overall study design provides the opportunity for diversity research along this management gradient across Germany (for further information see Fischer et al. 2010).

1.2 **Thematic focus and model organism**

At the basis of ecosystems, detritivores provide valuable services, for example the utilization and processing of organic materials, which otherwise would remain inaccessible for higher trophic levels. For example by yielding nutrients or as prey, these organisms are an important part of ecological recycling (Orgiazzi et al. 2016).

The present thesis is a project, which was established and conducted within the Biodiversity Exploratories and combines the applied and basic research for a basal superfamily of such detritivorous insects: dung beetles.

Dung beetles (Coleoptera; Scarabaeoidea) are widespread in most habitats around the globe and represent important ancient and current detritivores (Hanski and Cambefort 1991, Chin and Gill 1996). Beyond the global distribution of this superfamily, geographical regions and climatic zones show characteristic spectra of dung beetle communities. While north temperate and cooler regions favor the presence of *Aphodius* and Geotrupidae species (Halffter and Matthews 1966), Scarabaeidae (esp. *Onthophagus* and some *Aphodius*) abound in tropical regions, including hot spots with more than 80 species that have been found locally in tropical forests and savannahs (Hanski and Cambefort 1991, Davis 2000, Feer and Hingrat 2005, Barragan et al. 2011). In Central Europe, however, all three families (Geotrupidae, Scarabaeidae and Aphodiidae) occur in high abundance (Fig.1.2). Due to the historical and recent central European climate, the distribution of genera shows a (taxonomically) diverse community structure, compared to northern and southern temperate zones (Hortal et al. 2011).

Furthermore, dung beetles show distinct habitat preferences (Hill 1996, Davis et al. 2001). Communities change across habitats, and a complete species turnover has been observed within natural ecotones (Spector and Ayzama 2003). Also, dung beetles respond to the overall vegetation structure from forest to grassland (Nummelin and Hanski 1989, Davis et al. 2001) and to disturbances such as habitat fragmentation or timber harvest, for which particularly strong effects on "habitat specialists" (Halffter and Arellano 2002) and negative impacts on species richness and abundance were found (Howden and Nealis 1975, Hanski 1989, Klein 1989, Estrada et al. 1999).

Figure 1.2: Three genera of dung beetles. Left to right: *Anoplotrupes stercorosus* (Geotrupidae), *Onthophagus coenobita* (Scarabaeidae) and *Aphodius prodromus* (Aphodiidae). Additionally, these species represent two functional groups: Tunnelers (Geotrupidae, Scarabaeidae), which provide the majority of dung removal storing brood balls in their nests, and Dwellers (Aphodiidae), which primarily develop within larger dung pats.

Since dung beetles are relatively straightforward to sample, using dung baited pitfall traps, and they are a highly effective indicator species used in many studies (Hutton and Giller 2003, Spector 2006, Scholtz et al. 2009, Nichols and Gardner 2011). Although numerous studies in Europe focused on dung beetles as biological indicators with regard to anthropogenic influences (Martín-Piera and Lobo 1995, Hutton and Giller 2003, Spector 2006, Zamora et al. 2007), only few studies analyzed the effects of grassland and forestry management intensity in the field (Roslin and Koivunen 2001, Barragan et al. 2011, Filgueiras et al. 2015). Livestock farms and wildlife enclosures, furthermore, provide

clustered habitats (due to the fact that animals are kept at site), but also make large amounts of resources available, which are essential for the beetles' life cycles. Dung beetles are known to use a wide range of dung types among a few other resources such as carcasses, humus and fungi but also to specialize on their main resource: dung (Hanski and Cambefort 1991). The strong dependency on dung suggests that species-dependent preference among different dung types may occur (Yasuda 1987, Whipple and Hoback 2012b). Yet, there is little information about how land use alters resource selection in dung beetle communities. As mentioned before, (anthropogenic) disturbance affects the occurrence of species and drives changes in their behavior. Hence, those effects may result in a shift of species-resource interaction, not only for single species but also for the whole community. Such direct and indirect changes on different trophic levels (e.g. dung beetles processing dung, which enhances the nutrient input for plant biomass) can alter the community structure itself and in consequence their capability for ecosystem services.

1.3 **Aims and scope**

As land use, particularly in Europe, is highly diverse and differs in management activity and intensity (Herzog et al. 2006), investigation of variable land-use conditions on local (dung beetle) communities can be suitable to assess changes and predict consequences for species communities (Newbold et al. 2015).

Therefore, I assessed (1) the abundance, diversity and species composition of dung beetles in forests and grasslands, and their habitat-specificity, (2) how gradual variation in land-use intensity within grasslands and forests affects each of the dung beetle species, their interaction with resources and whether these responses can be explained by variation in dung specialization and (3) the preferences and removal across different dung types in the field. The assessment of these parameters, in context of a constantly managed environment, provides an applied and ecosystem-service related approach to monitor land-use intensity and its consequences for biodiversity on a detritivorous level.

Additionally, for a more basic understanding of the beetles' behavior and which drivers affect this environmentally valuable group of insects in its choice for certain recourses, I conducted (4) nutritional analyses of different vertebrate dung types and (5) dung beetle samplings with volatile organic compounds.

1.4 **Outlines**

Chapter 2 – *Land use affects dung beetle communities and their ecosystem service in forests and grasslands*

Tunneling genera like *Geotrupes*, *Anoplotrupes* and *Onthophagus* are known to enhance the soil quality and support nutrient cycles by rapid burial of mammalian dung. I used dung from livestock (cow, sheep, horse) and game animals (wild boar, red deer and fox) to provide a characteristic spectrum of dung resources and sampled 300 sites within three different areas in Germany, including forests and grasslands. I found distinct habitat preferences for different genera: Geotrupidae (*Anoplotrupes* and *Typhaeus*) and several *Aphodius* species almost exclusively occurred in forests, while most *Onthophagus* individuals were found in grasslands. Within this study I found the average abundance of dung beetles (converted to total biomass) is higher in forests than in grasslands and consequently, an enhanced dung removal in forests. For rising land-use intensity, I found dung beetle abundance and dung removal to be increased with grazing and in coniferous forests, but foremost a decrease of the beetles' removal activity by mowing, fertilization and wood harvest.

Chapter 3 – *Complexity of trophic networks improves the robustness of ecosystem functioning against land-use intensity*

As the connectivity and complexity of trophic networks is predicted to stabilize species communities and improve ecosystem functionality, I sampled dung beetles (18,770 individuals from 34 species) in 300 forest and grassland sites, to conduct an indepth network analysis of current dung beetle communities. I found that the sampled beetle species were relatively similar and highly generalized in their usage of dung types. Overall, higher complexity and generalization of dung beetle networks translated into a higher decomposition rate and a more balanced decomposition of different dung. Additionally the enhanced network complexity in forests (compared to grasslands) resulted in a three-fold increased and more even dung decomposition rate. Surprisingly, land-use intensity had little impact on network complexity and generalization in forests and grasslands, and larger species remained relatively tolerant, which contributes to an unexpected robustness against land-use intensity.

Chapter 4 – *Global dung webs: high trophic generalism of dung beetles along the latitudinal diversity gradient*

Species diversity on a global scale is known to increase towards the equator. In consequence, theory predicts a higher specificity of species (i.e. resource specialists) enabling the coexistence of species in more diverse communities. For several guilds and trophic levels these assumptions have been tested, but unexpectedly only few studies confirmed the predicted increase. Yet, analyses on a detritivorous level are still missing. Hence, I used 41 studies with a total of 990449 individuals summarizing data from 26 countries (6 continents) to calculate the dung beetle-resource specialization across the latitudinal diversity gradient on a global scale. The analyses confirmed rising beetle diversity towards the equator, yet the dung beetle-resource specificity remained generalistic. Hence, albeit having highly specialized species (and local communities), global patterns reveal a highly generalistic use dung types on the latitudinal gradient.

Chapter 5 – *Nutrient quality of vertebrate dung as a diet for dung beetles*

Dung beetles showed significant preferences for certain dung types and several studies have characterized the nutrients (C/N ratios and organic matter content) of specific dungs. A comparative approach, however, across dung types and relationships between dung nutrients and the beetles' preference, has been missing. Therefore, I analysed water content, C/N ratio, amino acid, neutral lipid fatty acid, free fatty acids and sterol composition and concentrations in dung collected from 23 vertebrate species, including carnivores, omnivores and herbivores. The three vertebrate feeding guilds had significant differences for most nutritional parameters, yet dung appeared to contain sufficient amounts of essential nutrients for insects. In a field experiment I tested whether nutrients can explain the dung beetles' preferences, using 12 representative dung types that were installed in 27 forests and 27 grasslands. Despite consistent preferences for specific dung types, the nutritional composition did not predict the variation in dung attractiveness, suggesting a primary role of dung volatiles irrespective of food quality.

Chapter 6 – *Dung beetle attraction and the meaning of volatile composition in dung*

Dung beetles rely on scenting resources, yet the role of volatile organic compounds (VOCs) for dung localization and differentiation among different resources remains open. In this study, I used six single chemical components (indole, skatol, phenol, butyric acid, 2-butanon and p-cresol), two VOC blends and six different dung types to disentangle dung beetle attraction towards a variety of different scents. Dung and chemical baits were able to attract similar communities, however there are large differences in the number of attracted species and their abundance. The analysis of dung scent profiles revealed unique patterns in composition and ubiquitous components, such as p-cresol. This might highlight the usage of key VOCs, but moreover that dung beetles use a mixture of components for resource localization.

Chapter 2

2 Land use affects dung beetle communities and their ecosystem service in forests and grasslands

Kevin Frank, Marietta Hülsmann, Thorsten Assmann, Thomas Schmitt and Nico Blüthgen

2.1 Abstract

Dung beetles (Scarabaeidae) are common detritivores, and especially the tunnelling genera *Geotrupes*, *Anoplotrupes* and *Onthophagus* enhance the soil quality and support nutrient cycles by rapid burial of mammalian dung. These functionally important beetles are faced with a wide range of anthropogenic disturbances and changes in environmental conditions due to land use. We thus conducted quantitative surveys of the abundance (converted to total biomass) of dung beetles and their dung removal rates (g per two days) in 150 forest and 150 grassland sites with varying land-use intensity, located in north-east, central and south-west Germany. We used dung from livestock (cow, sheep, horse) and game animals (wild boar, red deer and fox) to provide a characteristic spectrum of dung resources on each site. Most dung beetle species showed habitat preferences: *Anoplotrupes*, *Typhaeus* and several *Aphodius* species almost exclusively occurred in forests, while most *Onthophagus* individuals were found in grasslands. In total we collected 18780 individuals from 33 species. The average dung beetle biomass was 36 times higher in forests than in grasslands, and their effective dung removal rate was 3 times increased. The beetles' total biomass was strongly correlated to their removal rates. In forests, the amount of wood harvesting significantly reduced dung removal rates by 20%, and mowing frequency (–7 %) and fertilisation (–4 %) had a significant negative effect in grasslands. Dung removal by beetles increased with grazing intensity (+6 %), however, and was higher in non-native coniferous forests (+22 %). Overall, our study demonstrates negative effects of habitat conversion from forest to grassland, and negative effects of land-use intensity within forests and grasslands on dung beetle activities.

2.2 Introduction

Fossil evidence suggests that dung beetles exist since the Mesozoic Era (Late Jurassic – Early Cretaceous) thus demonstrating that the usage of dung became an efficient strategy of resource acquisition in a very early stage of fauna evolution (Chin and Gill 1996, Davis et al. 2002, Nikolajev and Dong 2010). Furthermore the nearly cosmopolitan superfamily of Scarabaeoidea.are the only known invertebrates that store fecal material in tunnels (Vander Wall 1990). Despite choosing an unpredictable and patchy occurring resource, dung consumption grants sufficient nutrients for adults and beetle larvae (Philips 2011). Because of their tunnelling behavior, dung beetles increase the input of nutrients into the soil, benefit the vegetation (Nichols et al. 2008, Wu et al. 2011) and minimize potential breeding grounds of (pathogenic) pests (Fincher 1973, Ridsdill-Smith and Edwards 2011). Dung pads would remain much longer without dung beetle activity (Walters 2008), preventing growth of vegetation and therefore may result in wasted pastures up to two years (Anderson et al. 1984). Additionally the burial of dung causes soil aeration and access for water (Bornemissza 1960, Bang et al. 2005), and it decreases soil compaction (Manning et al. 2016).

Although dung beetles are considered as generalists regarding their resources, various reactions towards their preference for dung types have been shown (Hanski and Cambefort 1991). Whether it depends on the "host animals" diet (carnivore, herbivore, omnivore) (Halffter and Matthews 1966, Whipple and Hoback 2012b), nutrients (Whipple and Hoback 2012b), odour intensity (Scholtz et al. 2009) or differences in volatile organic compounds (Schmitt et al. 2004, Dormont et al. 2007) – dung beetles are attracted to a wide range of different dung types, but in variable numbers (Whipple and Hoback 2012b). In spite of their ubiquitous presence, several dung beetle species are habitat-specific, and forests and grassland communities differ substantially (Roslin and Viljanen 2011). The beetles' sensitivity to disturbances varies across species, rendering dung beetles as suitable biological indicators often considered in monitoring programs, supported by the fact that their sampling is very simple and efficient (Scholtz et al. 2009). Several authors surveyed the diversity of dung beetles in response to land use in tropical ecosystems, where they show their highest diversity in forests and savannas (Hanski and Cambefort 1991, Estrada and Coates-Estrada 2002, Feer and Hingrat 2005, Hanski et al. 2007, Nichols et al. 2007, Barragan et al. 2011). In Europe, anthropogenic influences on the diversity and occurrence of dung beetles have also been monitored (Martín-Piera and Lobo 1995, Hutton and Giller 2003, Spector 2006, Zamora et al. 2007), highlighting changes for certain regions and habitats, such as grasslands and shrubs

versus planted forests (Romero-Alcaraz and Ávila 2000; Tocco et al. 2013). However, management activities are particularly diverse in European cultural landscapes, including different silvicultural management types, farm types (conventional versus organic), production systems (cropland, grassland, fertilisation and livestock) and socio-economic conditions (Reidsma et al. 2006). Apart from a differentiation in the type of land use, their quantitative intensities are strongly variable (Herzog et al. 2006). Reviews at the global scale (Newbold et al. 2015) thus highlighted the importance of local-scale studies for suitable characterisation and projection of land use on (local) biodiversity and ecosystem services (Allan *et al*., 2015; Soliveres *et al*., 2016).

In our study, we thus focused on such local effects of continuous land-use intensity gradients in forests and in grasslands on dung beetle abundance and ecosystem functioning. In addition to land-use gradients within forests or grasslands, we explicitly compared forest versus grassland, representing the two most common habitat types apart from arable fields and reflecting the historical habitat conversion from unmanaged or managed forest, originally covering vast parts of Central Europe, to cultivated grassland. In particular, we assessed (a) the abundance (biomass) of dung beetles, which are potentially involved in removal of various types of dung, and (b) the dung removal rate by these beetles within their habitats. Our goal was to quantify (1) how forests and grasslands differ in dung beetle biomass and their removal activities, (2) how habitat-specific, gradual variation in land-use intensity affects these beetles and their removal rates and (3) to understand which components of land use are responsible for this variation.

2.3 **Material and Methods**

Study Site

We conducted our study within the framework of the Biodiversity Exploratories project, comprising a large number of representative forest and grassland sites in three regions (north-east, central and south-west Germany) (Fischer et al. 2010). These sites varied continuously in land-use intensity, which was quantified based on farmer interviews and forest surveys. The three regions are: (1) Biosphere Reserve Schorfheide-Chorin (SCH; in North-East Germany, ~13.000 km^2, 3 – 140 m a.s.l., 13°23’27’’–14°08’53’’ E / 111 52°47’25’’–53°13’26’’ N), (2) Hainich National Park and surroundings (HAI; in Central Germany, ~13.000 km^2, 285 – 550 m 112 a.s.l., 10°10’24’’–10°46’45’’ E / 50°56’14’’–

51°22’43’’ N) and (3) Biosphere Reserve Schwäbische Alb (ALB; in South-West Germany, ~422 km^2, 460 – 860 m a.s.l., 09°10’49’’–09°35’54’’ E / 114 48°20’28’’– 48°32’02’’ N). Using a grid of 100 x 100 m placed over the entire area within each region, experimental plots (hereafter: sites) were chosen at random. Sites with inhomogeneous land cover or partial overlap with settlements, agricultural fields, water bodies and sites intersected by roads were discarded. In each region, 100 square-shaped sites were selected, 50 sites in forests (each 100 x 100 m) and 50 in grasslands (50 x 50 m), which are representative for the regional variation in land-use and management intensities. All sites are surrounded by a larger area of the same land use, i.e. the squares are usually only a small part of the forest or grassland with a specific management.

Our studies are based on two approaches:

(a) Comprehensive survey: a survey of all 300 experimental sites during summer 2014 was conducted once to maximize spatial replication.

(b) Intensive survey: on a subset of 54 of these sites (9 forests and 9 grasslands per region), we repeatedly surveyed the dung beetles and their activity to account for temporal variation across seasons and years. Since the comprehensive survey includes these 54 sites, we additionally used this subset from summer 2014 (a) in the analyses of temporal variation.

For the comprehensive survey we sampled the 100 sites per region in 20 days (SCH – June, HAI – July, ALB – August) (10.06.14 – 04.07.14; 07.07.14 – 01.08.14; 04.08.14 – 29.08.14). For the intensive survey we sampled each region (starting in SCH, followed by HAI and then ALB) for 5 days each in May 2014, December 2014, April 2015 and July 2015 (05.05. – 23.05.14; 01.12. – 12.12.14, 06.04. – 24.04.15; 29.06. – 17.07.15). Days of sampling were constrained by field permissions (weekends excluded) and logistics (9 – 12 sites per day).

As we did not discover any beetles in December and registered no removal at all, we excluded the December survey from further analysis and results.

In each site we monitored the dung beetle abundance and dung removal simultaneously for 48 h. To assess dung beetle abundance, we used dung-baited pitfall traps. To account for the beetles characteristic spectrum of dung resources available, we used six different dung baits consisting of three livestock and three game species, namely: cow (*Bos taurus* L., 1758), horse (*Equus caballus* L., 1758), sheep (*Ovis aries* L., 1758), red deer

(*Cervus elaphus* L., 1758), wild boar (*Sus scrofa* L., 1758) and fox (*Vulpes vulpes* L., 1758). For removal rate experiments we used the same dung types (due to very low quantities of fox dung we were only able to use it for pitfall traps during the intensive survey). For both, pitfall traps and removal experiments, dung samples were collected from the same sources. Livestock dung was collected at the farm 'Oberfeld' in Darmstadt (cow and horse) and at a sheep farm in Darmstadt (sheep); all livestock animals were grazing in pastures for at least part of the day, cows were additionally provided hay. Game species dung has been collected in the wildlife park 'Alte Fasanerie' in Hanau (fox, wild boar and red deer) and at the zoo 'Opel-Zoo' in Kronberg (additional fox). Diets for the animals were as follows: cow: grazing on pasture, hay; horse and sheep: grazing on pasture; red deer: grass, hay, maize, fodder beet, lucerne pellets, apples, carrots; wild boar: pig food (Raiffeisen), bread, maize, fruit, vegetables, lucerne pellets, meat of cattle, fallow deer and red deer; fox: 60 % meat (chicken, mice, rats, cattle), fruits, vegetables. Veterinarian medication (e.g. Ivermectin) can influence the treated animal's dung and is known to have negative effects on dung beetle performance (Lumaret et al. 2012, Verdu et al. 2015). According to farmers and animal keepers, however, all animals involved in this study have not faced any veterinarian treatment for several weeks before dung collection. Therefore, we do not expect adverse reactions during removal activity. After collecting samples in a sufficient amount, the dung was prepared in a lab either by filling dung in a tea bag and transferring the bait in a freezer bag or by filling freezer bags directly with dung for removal experiments. Afterwards the freezer bags where hermetically sealed, weighed and labelled. They were stored in a freezer at -20 °C until use, in order to prevent microbial decomposition, moulding or possible dung beetle activity (if small dung beetles had been accidentally collected in the dung).

Experimental design

Pitfall traps and removal rate experiments were placed on each site. Six pitfall traps (six dung types) were placed in a transect along the site margin, and in parallel five dung samples for removal assessment on the opposite side. Both, traps and dung samples were randomised on each site and separated by a distance of 10 m. For pitfall traps we used plastic cups (Ø 9.5 cm, height: 10 cm, vol.: 500 ml) and inserted dome lids with a hole (Ø 3 cm) as a funnel. The baits consisted of tea bags (Rubin, size S, Burgwedel, Germany) filled with dung (approx. 35 g for each bait), which were attached to a skewer by an elastic strap. When placing the traps, we took care that they were at ground level and had no barrier for walking

beetles. The skewer was placed next to the pitfall, so that the inaccessible bait was approx. 10 cm above the center of the trap.

For removal experiments, we used dung with a fresh weight of approx. 220.7 (± 19.9) g of cow, 34.4 (± 3.8) g of horse, 50.5 (± 3.6) g of sheep, 32.6 (± 1.6) g of deer, 14.5 (± 1.4) g of fox and 47.6 (± 2.4) g of wild boar. All dung samples have been placed on cellulose paper. This method allowed us to verify whether missing dung can be assigned to dung beetle activity as we checked the paper for characteristic holes, which often occur by tunnelling species (*Geotrupes* and *Onthophagus*). We then calculated only the removal rate for those dung samples showing such holes or where the cellulose paper was destroyed. For other samples that may have missing amounts of dung (e.g. due to incomplete retrieval or activities of other animals), removal was set to zero. Furthermore we took only tunnelling species into account to analyse the correlation between beetle abundance in traps and removal per site. After 48 hours pitfall traps were collected, trapped beetles were labelled (date, site-ID, dung type) and stored in a freezer at -20 °C. Dung samples of removal experiments were also collected, transferred into small paper bags, labelled (date, site-ID, dung type) and stored in a freezer at -20 °C. Caught dung beetles were identified to species level based on literature (Freude et al. 1969, Bunalski 1999, Rössner 2012) and with the help of taxonomic experts (see Acknowledgements). Removal samples were transferred into drying ovens and kept there at 60 °C for at least five days. Afterwards the dry weight for each dung sample was weighed (Mettler Toledo "EL 2001" (± 0.01 g), Columbus, Ohio) and noted for further calculations.

Data analysis

To examine the sampling completeness of occurring dung beetle species in each region and habitat (forests and grasslands), we calculated the estimated species richness test based on the Chao 1 index.

Temperature data (in °C) were measured with sensor stations installed within the Biodiversity Exploratories project on each site. Annual temperature time-series were used, to obtain the mean temperature over the 48 hours dung/trap exposition time for each site at 10 cm above ground.

For land-use characterisation we used two habitat specific indices: land-use intensity (LUI) and forest management intensity (ForMI). LUI is based on grazing (*G*), i.e. the number of livestock units times the days of grazing per ha and year (see Fig. S5 for presence/absence

of livestock and type of grazing), fertilisation (*F*), kg nitrogen applied per ha and year, and the frequency of mowing (*M*) per year. All parameters (*G*, *F*, *M*) are evaluated on annual basis (interview with farmers), which results in an updated land-use intensity index for each year, respectively. Furthermore, all factors were standardised per site *i* for a given year, relative to its mean within the corresponding model region *R* for that year. To reduce the impact of outliers and achieve a more even distribution, a square root-transformation was applied to the LUI, which is thus described as follows:

$$LUI = \sqrt{\frac{G_i}{G_R} + \frac{F_i}{F_R} + \frac{M_i}{M_R}}$$

Due to standardisation by ratios, the LUI is dimensionless. For more details see Blüthgen et al. (2012).

The ForMI is based on three parameters as well: the ratio of harvested tree volume to the sum of standing, harvested and dead wood volume (I_{harv}; a value of 0 describes no timber harvest in the last 30-40 years, a value of 1 a clear-cut site); the volume proportion of tree species that are not part of the natural forest composition, estimated as proportion of wood volume of non-native tree species to the sum of wood volume of all tree species (I_{nonat}; a value of 0 is a stand composed of natural forest vegetation only, 1 means that the whole stand consists of non-native tree-species); and the proportion of dead wood volume showing signs of saw cuts to the total amount of dead wood volume (I_{dwcut}; a value of 0 describes that all dead wood is a result of natural tree death, 1 that all dead wood is originated from management activity). These three parameters are summarised as:

$$ForMI = I_{harv} + I_{nonat} + I_{dwcut}$$

As the shift in tree species composition described in I_{nonat} comprises mainly coniferous species (*Picea abies* and *Pinus sylvestris*) that are not native in the study sites, we focus on the proportion of conifers below. Index data were assessed during forest inventory in 2008 and 2009 for living stands, in 2012 for I_{dwcut} (recording all dead wood > 25 cm on the 1 ha site), while I_{harv} was calculated based on allometric functions (Muukonen 2007). Since all components are calculated as proportions, they are dimensionless. For more details see (Kahl and Bauhus 2014).

Due to the skewed distribution of the removal and biomass data we transformed each dataset to achieve normal distribution as described in Table 1. In addition we log-transformed the LUI index components (*G* and *F*) to avoid outliers in regressions.

Data were analysed with linear mixed-effects models (command 'lme') for the comprehensive survey and linear models (command 'lm') for intensive surveys, using the statistical software package R 2.15.3 (R Core Team 2013) including the package ´nlme´ (Pinheiro et al. 2014). As response variable in the model, we either used the mean dung removal rate across all dung types, or the total dung beetle biomass across all dung types; hence dung types were pooled per site. Region and habitat-specific land-use indices or their components were employed as fixed factors in the model. The interaction terms served to assess whether the land use effects were consistent across regions or not. Dung beetle activities are known to vary with air temperature (Dortel et al. 2013) like other insect groups such as pollinators in the same study region (Kühsel and Blüthgen 2015); to account for a potential temperature bias on dung beetle activities, we used temperature as a random factor. For the intensive surveys we used the factor 'month' rather than temperature, and site identity as random factor to account for replicated surveys. To summarise the responses of removal with increasing land-use intensity, we added slope values of linear regressions for each analysis. We estimated the per cent change along each gradient (full range from minimum to maximum land-use intensity) based on the slope and intercept from each model. When interaction terms were significant, we provided separate analyses for each region (Supplementary Material: Table S1 & S2).

Removal rates were defined by dry mass. By dividing dry weight by fresh weight we calculated the dry mass content (P_{DM}) for each dung type and sampling. This approach allowed us to estimate the dry mass removal for each dung sample placed in the field as:

$$removal\ (g) = \left(fw_{before} * P_{DM}\right) - dw_{after},$$

with fw_{before} being the fresh weight of a sample before exposition in the field and dw_{after} being the weight of the samples collected in the field after 48 h. To account for possible differences among collected dung types and collection dates, we determined water contents of 3 – 8 samples randomly selected samples for each dung type and each collection day and treated them as described above for removal samples, except that we did not expose them to the field. Average water contents (proportions) were for cow: 0.85 (± 0.01), sheep: 0.74 (± 0.01), horse: 0.69 (± 0.03), deer: 0.55 (± 0.02), fox: 0.41 (± 0.03), wild boar: 0.46 (± 0.02).

As the beetles' body size is correlated to their dung removal rate (Nervo et al. 2014), we translated the abundances of the beetles into biomass. We calculated the species-specific body mass, based on the mean dry weight in g (dried at 60 °C for 3 days) measured on a microbalance (Mettler Toledo "EL 2001" (± 0.01 g), Columbus, Ohio) for 3-10 randomly selected individuals per species across all regions. Species-specific dry mass was multiplied with the species' abundances per site to quantify the total biomass for further analysis.

2.4 **Results**

Dung beetle community

In the comprehensive survey, we sampled 8297 individuals from 26 species of dung beetles in 206 experimental sites, hence in 94 of the total 300 sites, no dung beetles were trapped. For the intensive survey we sampled 10483 individuals from 31 species in 54 experimental sites (each surveyed four times); the total number of species in all surveys was 33 (5 Geotrupidae, 10 *Onthophagus*, 18 *Aphodius* species). While the three most common species of the family Geotrupidae and 13 of the *Aphodius* species mainly occurred in forests and represent the vast majority of caught individuals, 8 species of the genus *Onthophagus* were predominantly found in grasslands (Fig. 2.1).

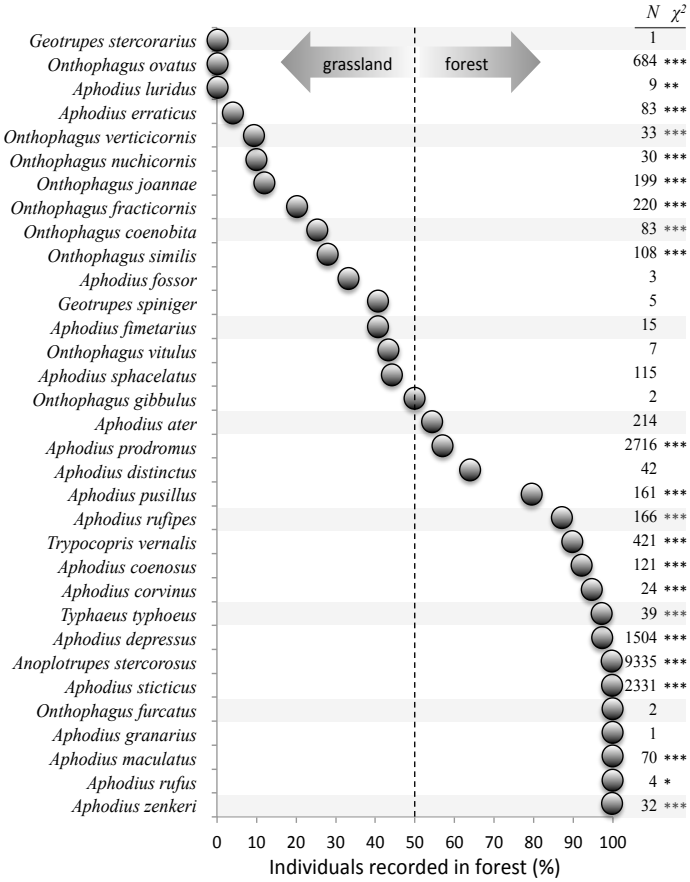


Figure 2.1: Distribution of dung beetles between forest and grassland sites, given as the proportion of individuals of each species found in forests. *N* is the total number of individuals in both habitats, and the asterisks provide the significance of a habitat preference (χ^2-test against 50% for all species with $N \geq 5$: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Overall, dung beetle biomass was 10 times higher in forests than in grasslands in the Alb (Welch t-test, $t = 3.83$, $p < 0.001$), 20 times in the Hainich ($t = 5.62$, $p < 0.001$), up to 80 times in the Schorfheide ($t = 8.99$, $p < 0.001$) (Fig. 2b). Our surveys represented the dung beetle species pool very well: for the comprehensive survey the dung beetle richness in forests showed ≥ 87.5 % sampling completeness when the recorded and estimated richness (Chao1) were compared in each region, and for grasslands the completeness was even 100 %. For the intensive survey richness in forests showed ≥ 85.7 % completeness, while the grasslands completeness was ≥ 86.9 %.

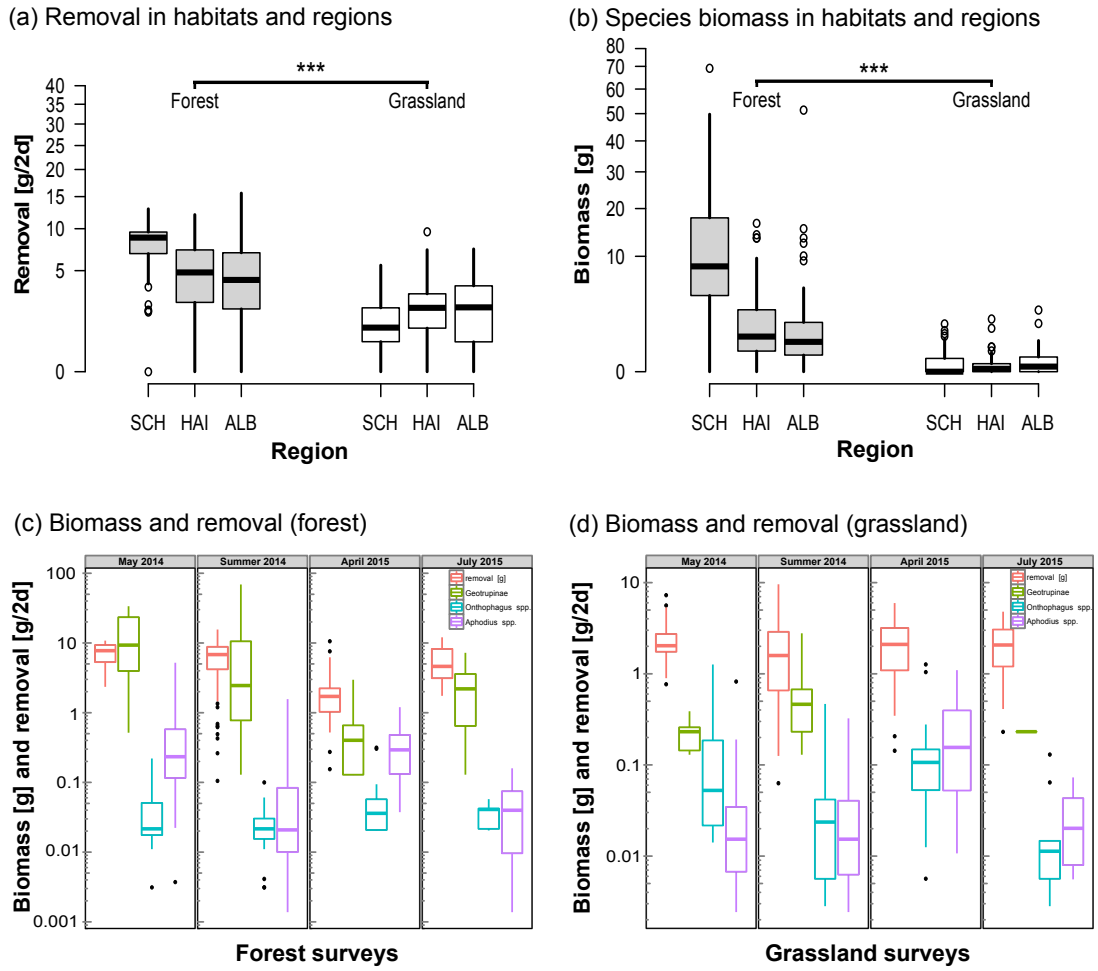


Figure 2.2: Overview for all surveys. Mean dung removal (a) and mean biomass of caught beetles (b) for each region and habitat and comparison of mean dung removal with mean biomass of caught beetles for each survey (c) and (d). Note that the y-axis of (a) is square root transformed, while the y-axis of (b), (c) and (d) is log-transformed.

In general, different management of the habitats showed contrasting effects on the beetles' biomass. While increasing by grazing intensity, the dung beetles' biomass decreased with the intensity of mowing and fertilisation in the comprehensive survey. For the intensive survey we found no significant effects, except at a regional level: only in the Alb, forests dominated by conifers had a higher dung beetles biomass (Table 1, Figs 3 & 4; Figs S1 & S2).

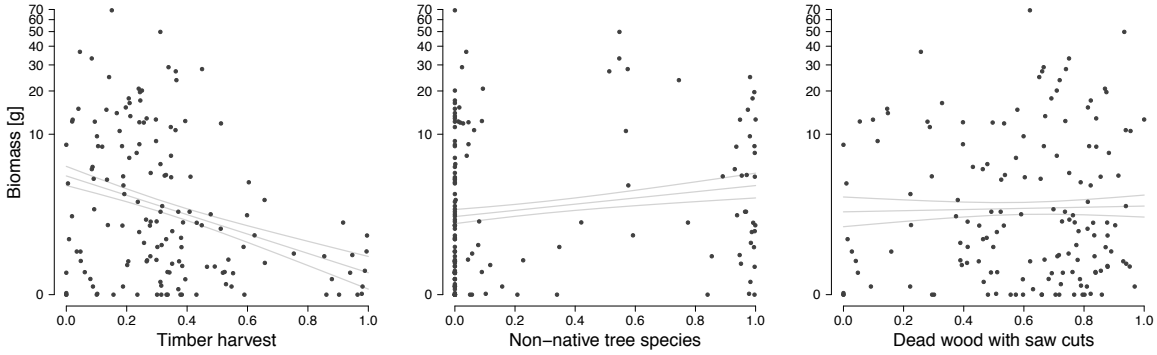


Figure 2.3: Biomass of individuals (g dry mass) collected during the comprehensive survey (n = 150 sites) plotted against components of forest management intensity index (ForMI): proportion of timber harvest, proportion of non-native tree species and proportion of dead wood with saw cuts. Note that the y-axis is log-transformed.

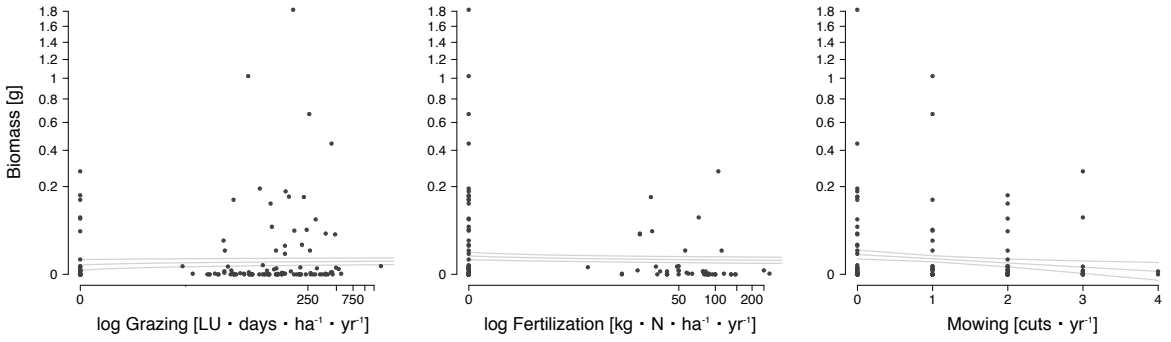


Figure 2.4: Biomass of individuals (g dry mass) collected during the comprehensive survey (n = 150 sites) plotted against components of land-use intensity index (LUI): grazing, fertilisation and mowing. Note that y-axis, grazing and fertilisation (x-axis) is log-transformed.

Table 2.1: Effects on overall dung removal rate (g dry mass per two days) and biomass (g dry weight) of dung beetles for (a) the comprehensive survey (n = 150 forests and 150 grassland sites) and (b) the intensive survey (n = 27 forests and 27 grasslands, four surveys each). Main effects (*F*-values) and significance (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) of linear mixed effect (LME) models are shown for habitat-specific indices (ForMI: Forest management intensity index, LUI: Land-use intensity index) and their components. The trend states the per cent increase or decrease of removal or biomass along the entire management gradient (from the minimum to the maximum gradient value), as predicted from the slope and intercept of the LME model.

			(a) comprehensive survey				(b) intensive survey			
			Removal [g/2d][1]		Biomass [g][2]		Removal [g/2d][1]		Biomass [g][2]	
Region	Habitat	Interaction	*F*	Trend	*F*	Trend	*F* [3]	Trend	*F* [3]	Trend
All	Forest	ForMI	0.3	+8.8 %	1.0	+7.0 %	0.0	+5.7 %	0.2	+32.2 %
		Region×ForMI	3.9*		2.4		3.6*		4.3*	
		TimberHarvest	5.3*	–19.6 %	1.4	–35.6 %	0.4	–0.7 %	0.5	+28.5 %
		Region×WoodHarvest	0.6		0.5		1.4		0.6	
		NonNativeTreeSpecies	6.5*	+22.3 %	3.4	+22.3 %	0.3	+13.6 %	0.9	+67.5 %
		Region×NonNativeTrees	6.3*		0.4		2.9		5.1**	
		DeadWoodWithSawCuts	0.2	+39.8 %	0.5	+39.8 %	0.0	+8.3 %	0.1	+55.3 %
		Region×DeadWood	0.8		2.6		1.7		1.9	
All	Grassland	LUI	0.3	–6.1 %	2.5	–83.3	6.4**	–10.4 %	1.8	+5.1 %
		Region×LUI	0.6		1.0		0.9		2.0	
		log Grazing	7.7**	+5.7 %	4.5*	+58.1 %	2.4	+1.35 %	0.4	–13.3 %
		Region×(log Grazing)	0.2		0.4		0.4		0.4	
		log Fertilisation	2.3	–2.8 %	5.6*	–20 %	8.5**	–4.2 %	2.0	+6.25 %
		Region×(log Fertilisation)	0.1		1.0		0.2		0.9	
		Mowing	5.9*	–7.2 %	8.9**	–41.6 %	3.2	–7.9 %	0.5	+26.6 %
		Region×Mowing	0.2		0.3		0.9		1.5	

1) data square root transformed
2) data log transformed,
3) As the factor 'month' is used to take account for multiple surveys, the lowest *F*-value of all surveys is listed.

Dung removal

Comprehensive survey: dung removal rates were 3.5 times higher in forests than in grasslands (Welch t-test, t = 16.27, p < 0.001) (Fig. 2a). Again, management showed contrasting effects: the proportion of non-native tree species (conifers) in forests and grazing intensity in grasslands as associated with an increase in dung removal (removal activity rises by +22.3 % from forests with no non-native trees to monospecific conifer stands, and by +5.7 % from no grazing to the maximum observed grazing intensity). The amount of timber harvest in forests and mowing events in grasslands, however, had significant negative effects, resulting in a decrease of removal activity by –19.6 % and –7.2 % along the range of these gradients, respectively (Table 1, Figs 5 & 6).

Intensive survey: dung removal rates were 2.7 times higher in forests than in grasslands (Welch t-test, t = 8.98, p < 0.001). Regarding the land use effects, there was a decrease in removal due to fertilisation in grasslands (by –4.2 %), whereas no significant effects were found in forests (Table 1, Fig. 6, Figs S3 & S4).

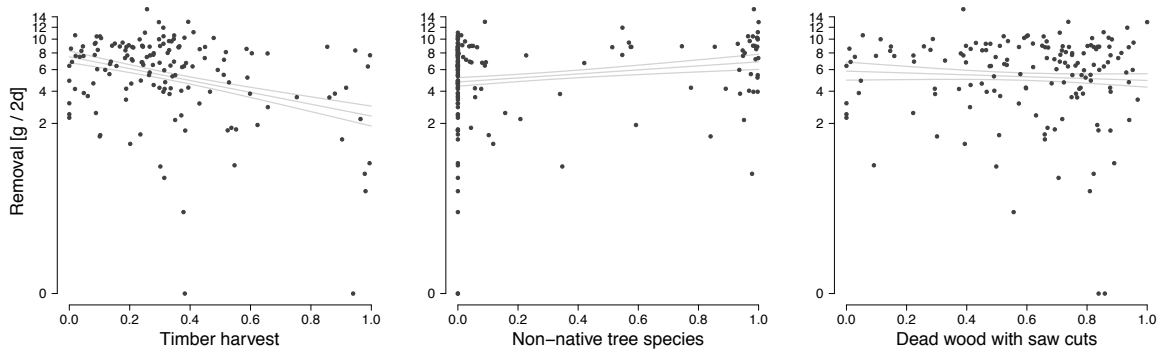


Figure 2.5: Mean dung removal (g dry weight per two days) on forest sites (comprehensive survey, n = 150) plotted against the components of forest management intensity index (ForMI): proportion of timber harvest, proportion of non-native tree species and proportion of dead wood with saw cuts. Note that the y-axis is square root transformed.

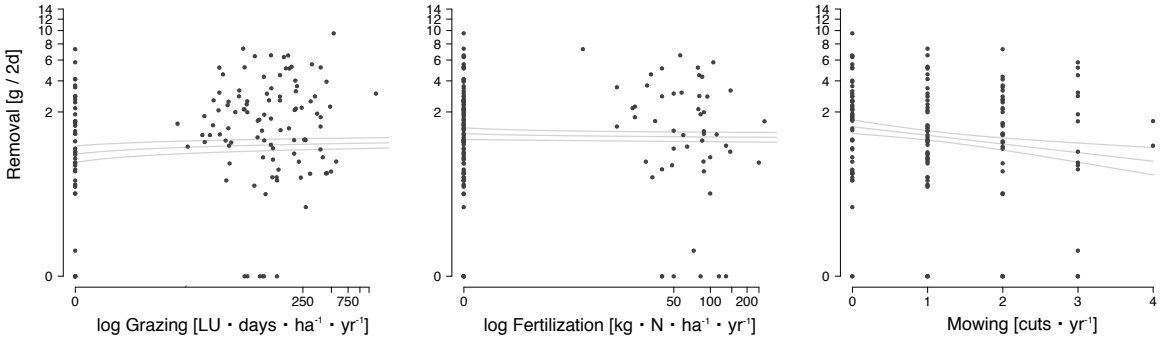


Figure 2.6: Mean dung removal (g dry weight per two days) on grassland sites (comprehensive survey, n = 150) plotted against the components of land-use intensity index (LUI): grazing, fertilisation and mowing. Note that the y-axis is square root transformed, while grazing and fertilisation (x-axis) is log-transformed.

Dung removal was highly correlated with biomass of caught beetles in pitfall traps of the same sites (Spearman rank test: $r_S = 0.78$, $p < 0.001$; all surveys combined). The increase in removal rate per additional beetle biomass was 0.52 g/g (slope of the log(removal+1) / log(biomass+1) relationship, $r^2 = 0.47$). For both surveys, removal on pastures did not differ among types of grazing (sheep, horse, cattle) (ANOVA, $F_{1, 270} = 2.17$, $p = 0.14$).

2.5 **Discussion**

Dung beetles are important ecosystem service providers, but are also known to negatively respond to anthropogenic disturbances and land use across the globe (Nichols *et al.*, 2007). Our study confirmed land-use effects on dung beetle activity and their ecosystem service in Central European cultural landscapes. In this region, many centuries of intense human activities have led to habitat conversion from forest to cultivated grassland and/or arable land. Forests – irrespective of their management – had several times higher beetle densities and dung removal rates than managed grasslands. While coniferous forests had significant positive effects on removal, timber harvest showed negative effects. In grasslands, beetles and their activity were positively affected by livestock densities, whereas mowing and fertilisation caused a decline of the beetles' biomass and removal activities. Whereas the difference between forests and grasslands was pervasive, the continuous land-use intensity gradients within forests or grasslands showed much weaker trends. Significant gradual responses were detected only in the comprehensive survey, but not confirmed in the intensive survey on a subset of the sites except the negative effect of fertilisation on removal. Therefore, a high number of spatially independent replicates is important for detecting such within-habitat responses.

Our assessment of dung removal within two days predominantly reflected the activities of the tunnellers, i.e. the family of Geotrupidae and the genus *Onthophagus*. Larger time scales of several weeks, up to months, give additional taxa the opportunity to contribute in removal activity, which highlights the complexity of multiple services for ecosystem functioning (Nervo *et al.*, 2015). Still, we focused on the two tunnelling taxa as they primarily bury dung (Anduaga 2004, Walters 2008) and are responsible for most of the removal within a short time frame of two days (Nervo et al. 2014). Additionally, the tunnellers represented 60 % of the dung beetle individuals collected and 96 % of the overall beetle biomass.

Habitat preference

Across the regions we found clear indications of habitat differentiation. The tunnelling species *Anoplotrupes stercorosus* was almost exclusively caught in forests where it was the most dominant species. Moreover, *Trypocopris vernalis* mostly occurred in coniferous forests. Conversely, *Onthophagus fracticornis, O. joannae* and *O. ovatus* constituted the majority of Scarabaeinae in grasslands. Peyras et al. (2013) highlighted distinct species-specific responses towards habitat fragmentation for dung beetles. In addition – depending on species, resource distribution and environmental conditions – habitat preferences can lead to distinct spatial mosaics of dung beetle communities and edge effects at habitat margins (Ries and Sisk 2004). As dung beetles typically utilise fresh dung piles which emit higher amounts of volatiles and provide suitable consistence for handling, the exposition of small dung piles on unshaded grasslands can cause higher dehydration and thus result in lower attractivity and removal. Since beetle abundance, biomass and removal rates were strongly correlated (consistent with the findings by (Tixier et al. 2015), the lower beetle densities in grasslands corresponded to a much lower removal compared to forests. In consequence, tree removal and open canopies can be crucial for a substantially reduced dung removal service.

Land use and forest management

Depending on different landowners, regulations and goals, forest management and thus the level of disturbance differs substantially. Unlike the regular annual management activities in grassland, however, harvesting in forests is typically followed by long periods of regrowth; it thus seems likely that mobile dung beetles follow the forest recovery and succession. In fact, the spatial distribution of the dung-producing mammals determines the dung beetles occurrence (Kuhn 2010), and the beetles are able to sense dung volatiles at a great distance (Hanski and Cambefort 1991). Rembialkowska et al. (1982) described the use of foliage and coniferous litter for brood balls in *Geotrupes stercorosus*, highlighting the ability of dung beetles to switch to other resources when dung is scant. By this, and because of the dung beetles shift from saprophagy to coprophagy (Cambefort 1991b), forests provide additional resources besides dung, sustaining the survival of locally high numbers of beetles. Large areas of fir and pine forests, often planted after the second world war for quick reforestation and timber (Elling 2007), can sustain particularly high densities of *Anoplotrupes* e.g. in the Schorfheide. Such coniferous forests may be particularly attractive for game species by providing a more sheltered habitat, but also because of its continuous management and a strong development of understory vegetation (e.g. ferns, raspberries, wild strawberry,

blueberries) that serves as food for deer. As there is a strict management regime for game species in Germany, furthermore, there is strong anthropogenic pressure on the distribution of wildlife, either by physical barriers, such as fences, or by hunting (Arnold et al. 2015). In consequence, attractiveness of certain habitats and regions for dung beetles may respond to local game species populations. For the comprehensive survey, we showed management effects on dung removal rates in forests, yet no effects on dung beetle biomass. Because of the sheer abundance and widespread distribution of *Anoplotrupes stercorosus* (representing 94% of all tunnelling specimens in forests), the functional contribution of this single species may largely compensate for losses of some less abundant species.

In pastures, the density of livestock units per hectare has a positive effect on dung beetle activities, most likely driven by a higher availability of dung. Although intensive grazing can reduce dung beetle abundance in Ireland (Hutton and Giller 2003), the net effect was positive in our study (Table 1, Fig. S5 for livestock presence/absence on sites). On the other hand, heavy disturbances such as mowing might disrupt the establishment of dung beetle communities. Furthermore, pastures without permanent grazing (e.g. hayfields) offer poor conditions due to insufficient resources and by this are incapable to balance adverse conditions like higher temperatures (drought stress) and increased predation risk. Since different dung beetle species do have seasonal niches (Figs 2c and d) (Waßmer 1994), depending on the management, pastures may lack of attractiveness during different seasons and by this might cause the absence of certain taxa. Fertilisation and mowing are usually coupled, highly correlated and thus difficult to disentangle in their effects on dung beetles. Enhanced fertilisation results in higher productivity and increasing mowing frequency, yet another trade-off to species with decreased tolerance towards stress.

Conclusion

We were able to show on a species level habitat preference for dung beetles in a large-scale framework in three regions across Germany. Habitat differentiation has been described in the past, but there is a lack of information why certain dung beetle taxa differ in such great numbers between forests and grasslands (e.g. for the Schorfheide, 80 times higher beetle densities were found in forests than in grasslands). The strong habitat preferences among different genera suggest that there are other important environmental drivers besides anthropogenic influence, such as climatic conditions or soil quality, which seems incentive for further investigations.

For the comprehensive survey we were able to show broad effects of grassland and forest management intensity, affecting dung beetles and their ecosystem service negatively, likewise in forests and grassland. Since our analysis showed contrasting local effects – proportion of non-native tree species versus timber harvest, grazing versus fertilisation and mowing – dung beetle activities were relatively balanced along the aggregate land-use intensity gradients (ForMI, Kahl & Bauhus 2014 and LUI, Blüthgen et al. 2012) (Figs S6 and S7), and impacts are complex. Dung beetles seem to be able to compensate certain amounts of gradual disturbance by abundance, mobility or seasonal occurrence. However, as our study was conducted in regions that include protected areas and relatively heterogeneous landscapes, not dominated by intensive arable land, many of the broad risks for dung beetle communities are not covered. Major disturbances and regime shifts, particularly deforestation or conversion to arable land, pesticide application or lactone treatments of livestock are likely to cause a much stronger decrease of dung beetle densities, resulting in declining removal activity (Nichols *et al.*, 2007; Newbold *et al.*, 2015).

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2.6 **Supplementary Material to Chapter 2**

Table S2.1: Comprehensive survey. Effects on overall dung removal rate (g dry mass per two days) and biomass (g dry weight) of dung beetles. Due to significant interaction terms of the main model (Table 2.1), additional analysis was conducted for corresponding index components; biomass responses had no significant interaction with region. Main effects (*F*-values) and significance (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) of linear mixed effect (LME) models are shown for habitat-specific indices (ForMI: Forest management intensity index, LUI: Land-use intensity index) and their components. The trend states the per cent increase or decrease of removal or biomass along the entire management gradient (from the minimum to the maximum gradient value), as predicted from the slope and intercept of the LME model. Each region analysis contains n = 50 plots.

			Removal [g / 2d][1]	
Region	Habitat	Interaction	*F*	*Trend*
SCH	Forest	ForMI	0.6	+1.2 %
		NonNativeTreeSpecies	0.1	+0.6 %
HAI	Forest	ForMI	4.1*	-7.54 %
		NonNativeTreeSpecies	2.3	-9.4 %
ALB	Forest	ForMI	2.3	+11.1 %
		NonNativeTreeSpecies	9.3*	30.9 %

1) data square root transformed

Table S2.2: Intensive survey. Effects on overall dung removal rate (g dry mass per two days) and biomass (g dry weight) of dung beetles. Due to significant interaction terms of the main model (Table 2.2), additional analysis was conducted for corresponding index components. Main effects (*F*-values) and significance (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) of linear mixed effect (LME) models are shown for habitat-specific indices (ForMI: Forest management intensity index, LUI: Land-use intensity index) and their components. The trend states the per cent increase or decrease of removal or biomass along the entire management gradient (from the minimum to the maximum gradient value), as predicted from the slope and intercept of the LME model.

			Removal [g / 2d][1]		Biomass [g][2]	
Region	Habitat	Interaction	*F*	*Trend*	*F*	*Trend*
SCH	Forest	ForMI	1.6	+6.2 %	2.6	-8.4 %
		NonNativeTreeSpecies	-	+7.3 %	0.6	-6.1 %
HAI	Forest	ForMI	3.2	-8.9 %	0.8	-12.6 %
		NonNativeTreeSpecies	-	-13.7 %	0.9	-27.6 %
ALB	Forest	ForMI	1.9	6.3 %	15.0***	+108.3 %
		NonNativeTreeSpecies	-	+14.8 %	25.1***	+150 %

1) data square root transformed, 2) data log transformed, ForMI: Forest management intensity index, LUI: Land-use intensity index

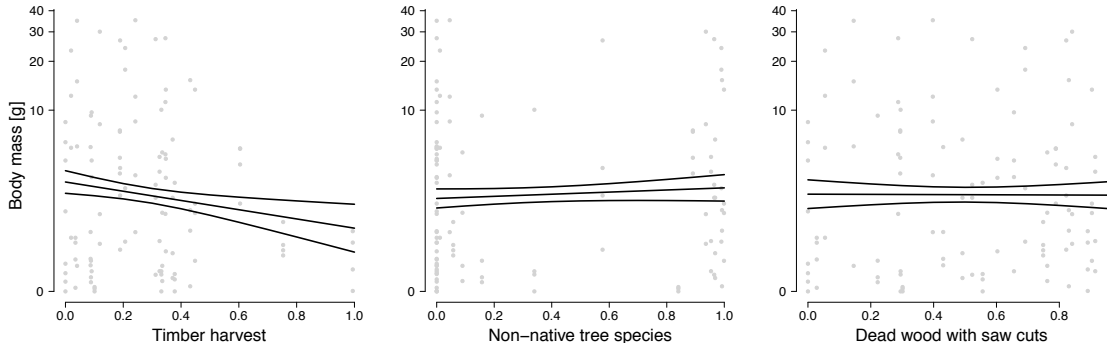


Figure S2.1: Biomass of individuals (g dry weight) collected on forest sites (intensive survey, n = 36) plotted against the components of forest management intensity index (ForMI). Note that y-axes are log-transformed.

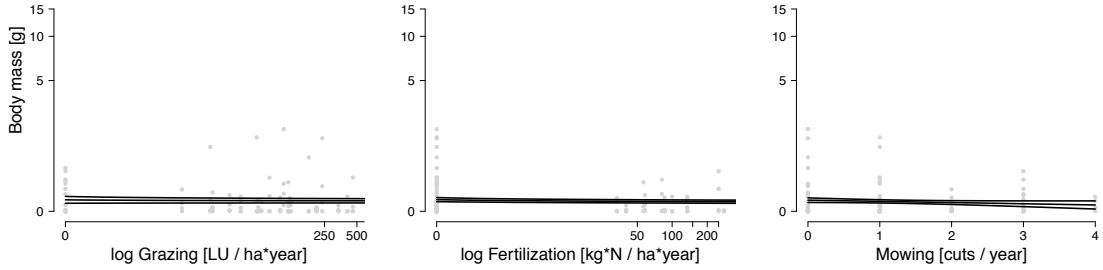


Figure S2.2: Biomass of individuals (g dry weight) collected on grassland sites (intensive survey, n = 36) plotted against the components of the land use intensity index (LUI). Note that y-axes are log-transformed

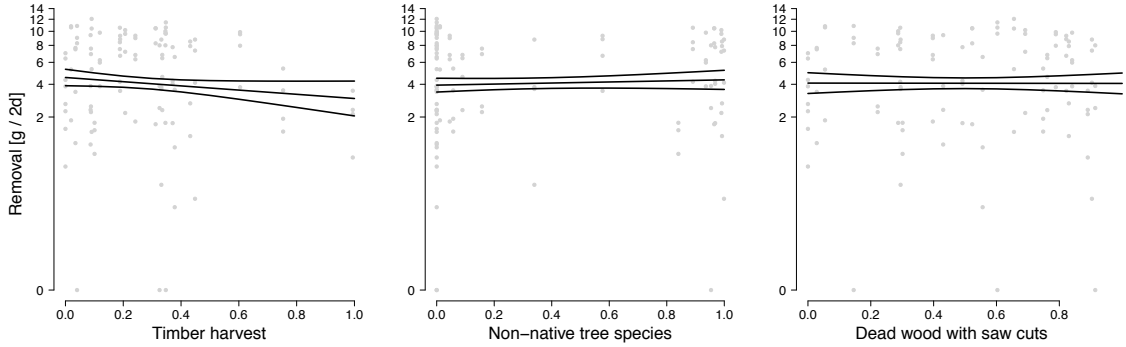


Figure S2.3: Mean dung removal rate (g dry mass per two days) on forest sites (intensive survey, n = 36) plotted against the components of forest management intensity index (ForMI). Note that y-axes are square log-transformed.

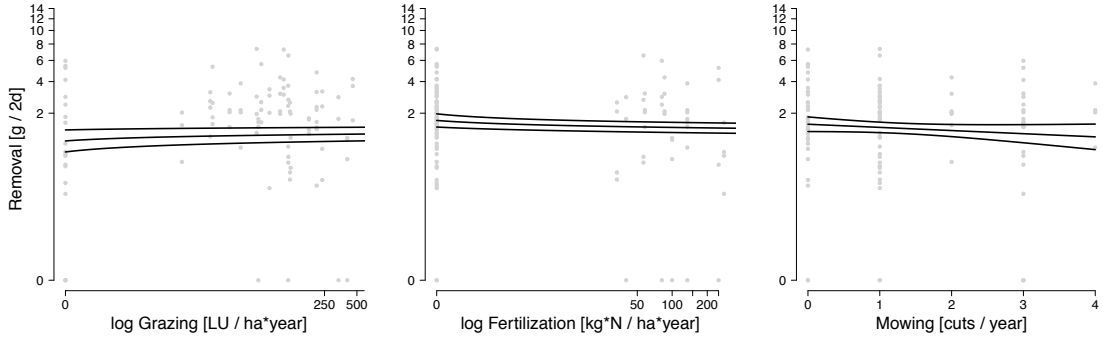


Figure S2.4: Mean dung removal rate (g dry mass per two days) on grassland sites (intensive survey, n = 36) plotted against the components of land use intensity index (LUI). Note that y-axes are square log-transformed.

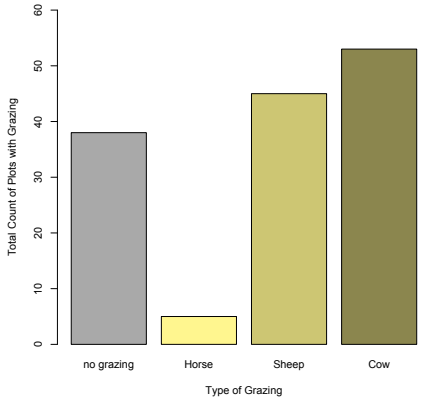
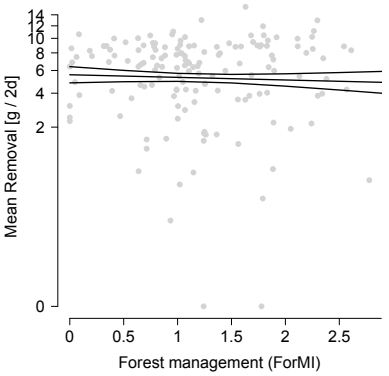
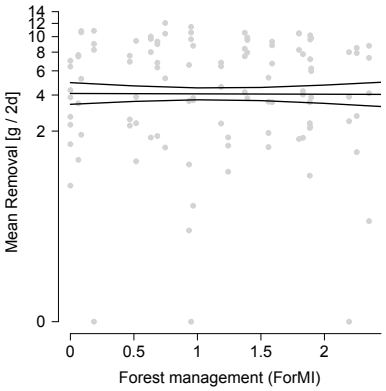


Figure S2.5: Comprehensive survey. Total count of sites with corresponding type of grazing livestock. Data states presence/absence of livestock for all 150 grassland sites.

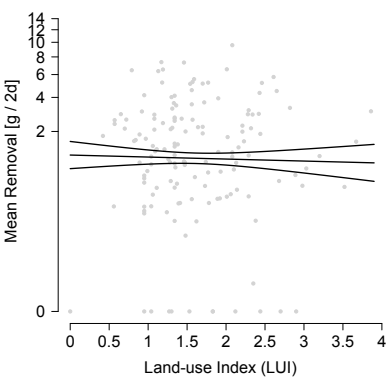
(a) comprehensive survey forest



(b) intensive survey forest



(c) comprehensive survey grassland



(d) intensive survey grassland

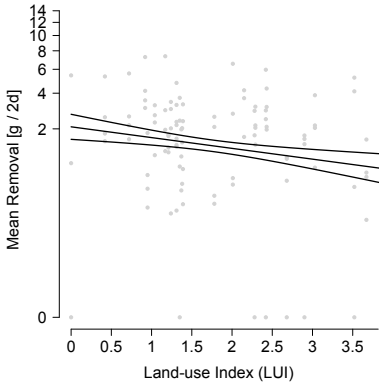
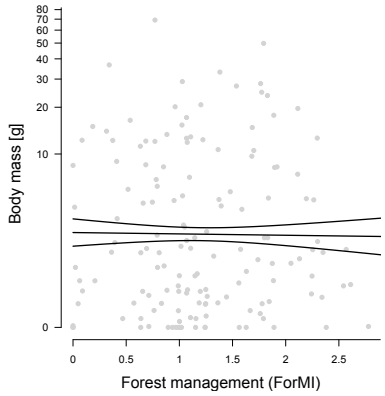
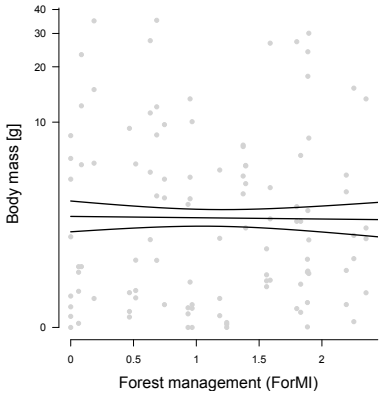


Figure S2.6: Mean dung removal rate (g dry mass per two days) in forest (comprehensive survey (a), intensive survey (b)) and grassland (comprehensive survey (c), intensive survey (d)), plotted against forest management intensity index (ForMI) and land-use intensity index (LUI). Note that y-axes are square root transformed. (comprehensive survey: n = 150; intensive survey: n = 108).

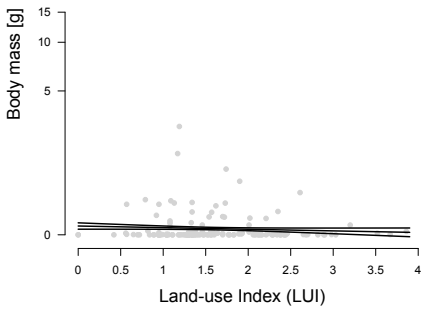
(a) comprehensive survey forest



(b) intensive survey forest



(c) comprehensive survey grassland



(d) intensive survey grassland

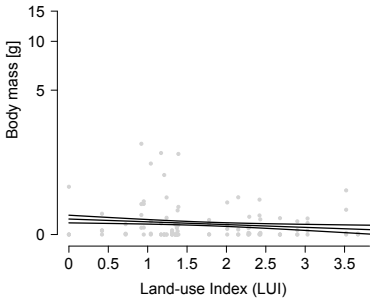


Figure S2.7: Mean biomass of caught individuals (dry weight in g) in forest (comprehensive survey (a), intensive survey (b)) and grassland (comprehensive survey (c), intensive survey (d)), plotted against forest management intensity index (ForMI) and land-use intensity index (LUI). Note that y-axes are log-transformed. (comprehensive: n = 150; intensive survey: n = 108).

Chapter 3






3 Improved functional performance and robustness from complex trophic networks

Kevin Frank and Nico Blüthgen

3.1 Introductory paragraph

The connectivity and complexity of trophic or mutualistic networks is predicted to stabilize species communities and improve ecosystem functioning (Macarthur 1955, McCann 2000, Dunne et al. 2002, James et al. 2012). However, this theory is controversial (May 1972, McCann 2000, Thebault and Fontaine 2010, Rohr et al. 2014) and poorly supported by empirical evidence in real-world ecosystems (Jacquet et al. 2016). Here we show that the degree of generalization of trophic interaction networks improves their functional effect in 300 forest and grassland ecosystems, which contributes to an unexpected robustness towards land-use intensity. We sampled 18,770 individuals from 34 dung beetle species and monitored their attraction to six common vertebrate dung types (networks) and simultaneously quantified dung removal rates. The total dung beetle abundance was 2 – 11 times higher in forests than in grasslands, associated with a higher diversity. Across sites, a higher complexity and generalization of dung–beetle networks translated into enhanced decomposition, which was more balanced across dung types and more stable across months. Networks in forests were more complex than in grasslands, resulting in a three-fold increased decomposition rate. Habitat-specific land-use intensity had little impact on networks. The high redundancy of trophic interactions, and particularly the tolerance of effective, larger species stabilized this important ecosystem service. The stability is maintained to some extent by species that replace each other along gradients, since individual dung beetle species showed contrasting responses to single types of land uses, including both significant positive (due to conifers or grazing) and significant negative effects (timber harvest or mowing).

3.2 **Main Text**

Most important processes in ecosystems are maintained by trophic, mutualistic, or antagonistic networks of interacting species. The diversity of species present in each community then provides the basis for networks made up of subsets of interactions and functions (Montoya et al. 2006, Rohr et al. 2014).

For almost half a century, the debate over the role of network complexity and diversity in ecosystem functioning and stability has been controversial (May 1972, McCann 2000, Allesina and Tang 2012). The complexity – stability relationship has largely been viewed through the lens of theoretical models of random food webs or mutualistic networks, models of extinction scenarios and conceptual arguments (Memmott et al. 2004, Rohr et al. 2014, Jacquet et al. 2016). Despite differing views (May 1972, Jacquet et al. 2016), several studies emphasized the positive effect of highly connected, complex networks and/or weak interaction for community persistence (Macarthur 1955, Dunne et al. 2002, Memmott et al. 2004, James et al. 2012), with potential consequences for the stability of functions performed by these species (although changes in functions have not been quantified). The proposed effects of the multidimensional complexity of interaction networks mirror the effects of species diversity (through their functional complementarity and redundancy) on ecosystem functioning and stability. Biodiversity has a dual effect on ecosystem functioning: (1) it increases the overall functional performance in a community, i.e. resulting in higher total process rates, due to functional complementarity of species, and (2) it buffers its temporal variability, due to statistical averaging (portfolio effect), species' asynchronies (insurance hypothesis) and complementary environmental responses of functionally equivalent species (response diversity of functionally redundant species) (McCann 2000, Elmqvist et al. 2003, Hooper et al. 2005, Tilman et al. 2006, Blüthgen et al. 2016). Biodiversity experiments with plants empirically confirmed the importance of functional complementarity of plant species to increase the overall productivity of a system. Furthermore, plant species richness improved the stability of productivity across years (Tilman et al. 2006). Consequently, losses of plant diversity due to anthropogenic disturbance translate into lower stability of productivity (Hautier et al. 2015). In general, a positive effect of biodiversity on ecosystem functionality may also occur if some species with a higher-than-average impact, such as larger species, are more likely to occur in species-rich communities, known as 'sampling' or 'selection effect' (Loreau and Hector 2001). The loss of certain species can cause a rapid functional decline, suggesting that ecosystems with a propensity to certain directional and non-random extinction orders, are particularly vulnerable (Larsen et al. 2005).

Trophic networks potentially translate the effect of biodiversity and species composition to ecosystem functioning, as the networks quantify the individual functional roles of species, how they overlap and complement each other (Montoya et al. 2006). It is likely that the same drivers also improve key processes in ecosystems other than plant productivity, such as particular mutualistic or trophic networks represented by predation, herbivory, pollination or decomposition. Only recently, it has been demonstrated that more generalized and complex pollinator networks in restored ecosystems enhance the pollination success of endemic plants on an oceanic island (Kaiser-Bunbury et al. 2017). Studies on the dynamics of species interaction networks have revealed important insights into anthropogenic effects and corresponding functional consequences (Tylianakis et al. 2007, Knop et al. 2017). However, for trophic networks a positive complexity – functioning relationship has been suggested but not yet been shown in real-world systems. Species-specific food-webs and decomposition are often difficult to track in the dark of the litter and soil or are lumped into larger resource types and trophic levels rather than species (Martinez 1991, Digel et al. 2014).

Dung beetles are found all over the world and are important detritivores, and their high consumption rate of vertebrate feces represents a valuable ecosystem service that can be easily quantified (Hanski and Cambefort 1991). The tunneling genera in particular bury vertebrate dung in a very short time. This enhances soil quality, supports nutrient cycles and thus increases plant productivity. In addition, they play an important role as dispersers of seeds contained in dung. The beetles' species-specific utilization of different types of dung can be characterized as a trophic network that aims to quantify the resource specialization and overlap across consumers that co-occur in space and time. To date, it is unknown how such dung–beetle networks respond to anthropogenic disturbances and changes in environmental conditions and how this variation translates into functional consequences.

Therefore we investigated the response of dung–beetle networks between and within forest and grassland ecosystems to different forms of land use. We asked whether changes in such trophic networks represent more subtle changes in its components – the total abundance, species diversity, resource specialization, consequences for individual beetle species – and how these changes translate into dung removal as an important ecosystem service.

3.3 **Material and Methods**

Study sites

This study was conducted within the framework of the Biodiversity Exploratories project, comprising three regions with representative forest and grassland sites in north-east, central and south-west Germany. These sites varied continuously in land-use intensity, which was quantified based on farmer interviews and forest surveys. The three regions are: (1) Biosphere Reserve Schorfheide-Chorin (SCH; in North-East Germany, ~13.000 km^2, 3 – 140 m a.s.l., 13°23'27''–14°08'53'' E / 52°47'25''–53°13'26'' N), (2) Hainich National Park and its surroundings (HAI; in Central Germany, ~13.000 km^2, 285 – 550 m a.s.l., 10°10'24''–10°46'45'' E / 50°56'14''–51°22'43'' N) and (3) Biosphere Reserve Schwäbische Alb (ALB; in South-West Germany, ~422 km^2, 460 – 860 m a.s.l., 09°10'49''–09°35'54'' E / 48°20'28''– 48°32'02'' N). In total, the study sites cover approx. 10% of Germanys agricultural land, pastures and forests. Within each region, 100 square-shaped experimental sites (hereafter: sites) were chosen at random, 50 sites in forests (each 100 x 100 m) and 50 in grasslands (50 x 50 m), which are representative for the regional variation in land-use and management intensities (i.e. all sites are part of a larger area of the same habitat and management type). Sites with inhomogeneous land cover or partial overlap with settlements, agricultural fields, water bodies and sites intersected by roads were discarded. For more details see Ref.(Fischer et al. 2010). Our study is based on two major surveys:

(a) Comprehensive survey: all 300 sites were sampled once to maximize spatial replication in June 2014 (SCH, 10.06. – 04.07.), July 2014 (HAI, 07.07. – 01.08.) and August 2014 (ALB, 04.08. – 29.08.)

(b) Intensive survey: on a subset of 54 of these sites (9 forests and 9 grasslands per region), we repeatedly surveyed the dung beetles and their activity to account for variation in occurrence across seasons and years. Since the comprehensive survey includes these 54 plots, we additionally used this subset from summer 2014. The intensive survey was conducted in May 2014 (SCH, 05.05. – 09.05.; HAI, 12.05. – 16.05.; ALB, 19.05. – 23.05.), December 2014 (SCH, 01.12. – 05.12.; HAI, 08.12. – 12.12.), April 2015 (SCH, 06.04. – 10.04.; HAI, 13.04. – 17.04.; ALB, 20.04. – 24.04.) and July 2015 (SCH, 29.06. – 03.07.; HAI, 06.07. – 10.07.; ALB, 13.07. – 17.07.). As we did not discover any beetles in December and registered no removal at all, we excluded the December survey from further analysis and results.

Temperature data (in °C) were measured with sensor stations installed within the Biodiversity Exploratories project on each site. Annual temperature time-series were used, to obtain the mean temperature over the 48 hours dung/trap exposition for each site at 10 cm above ground.

Experimental design

To assess dung beetle diversity, abundance and preference across different dung types, we used dung-baited pitfall traps. Six different dung types consisting of three livestock and three game species were used, namely: cow (*Bos taurus* L., 1758), horse (*Equus caballus* L., 1758), sheep (*Ovis aries* L., 1758), red deer (*Cervus elaphus* L., 1758), wild boar (*Sus scrofa* L., 1758) and fox (*Vulpes vulpes* L., 1758). Livestock dung was collected at the farm 'Oberfeld' in Darmstadt (cow and horse) and at a sheep farm in Darmstadt (sheep). Game species dung was collected in the wildlife park 'Alte Fasanerie' in Hanau (fox, wild boar and red deer) and at the zoo 'Opel-Zoo' in Kronberg (additional fox). Nutrient composition of these dung sources were reported elsewhere (Frank et al. 2017a). To prevent compromising baits due to medication (Lumaret et al. 2012, Verdu et al. 2015), we ensured no medical treatment for several weeks before dung sampling (interviews with farmers and animal keepers). For each bait, dung was filled in a tea bag (Rubin, size S, Burgwedel, Germany) and transferred in a freezer bag. Afterwards the freezer bags where hermetically sealed, labeled and stored at -20 °C until use, to prevent microbial decomposition, moulding or possible dung beetle activity (if small dung beetles had been accidentally collected in the dung).

To assess dung beetles, we used six dung-baited pitfall traps per site, each equipped with one of the six dung types; beetles were unable to reach the dung. In parallel, we used dung piles placed on soft tissue on the ground to measure dung burial by beetles; burial by beetles was indicated by holes in the tissue (we only accounted for dung removal if this was clearly attributable to beetles). On each site, we thus placed six pitfall traps and five or six dung piles for removal rate experiments; due to low quantities we had to exclude fox dung for removal in the comprehensive survey but not in the intensive survey. Traps and dung piles were placed in parallel transects, 50 m apart from each other. Within each setup the dung types were placed randomly by a distance of 10 m. The traps were made of plastic cups (vol.: 500 ml) with inserted dome lids as funnels and tea bags filled with dung (approx. 35 g for each bait), attached to a skewer by an elastic strap. We placed the traps at ground level and

took care that there was no barrier for walking beetles. Each bait was inaccessible approx. 10 cm above the center of the trap. In each site experimental setups remained for 48 h.

Trapped beetles were collected, labeled (date, site-ID, dung type) and stored in a freezer at -20 °C. In the lab dung beetles were identified to species level based on literature (Freude et al. 1969, Bunalski 1999, Rössner 2012) (Freude et al. 1969, Bunalski 1999, Rössner 2012)(Freude et al. 1969, Bunalski 1999, Rössner 2012)(Freude et al. 1969, Bunalski 1999, Rössner 2012)(Freude et al. 1969, Bunalski 1999, Rössner 2012)(Freude *et al.* 1969; Bunalski 1999; Rössner 2012) and with the help of taxonomic experts (see Acknowledgements).

For removal data we collected the remaining dung in the field and transferred it into drying ovens at 60 °C for at least five days. Afterwards the dry weight for each dung sample was weighed (Mettler Toledo "EL 2001" (± 0.01 g), Columbus, Ohio) and noted for further analyses. By dividing dry weight by fresh weight (of unused dung samples) we calculated the dry mass content (P_{DM}) of dung. This approach allowed us to estimate the dry mass removal for each dung sample placed in the field as:

$$removal\ (g) = \left(fw_{before} * P_{DM}\right) - \ dw_{after},$$

with fw_{before} being the fresh weight of a sample before exposition in the field and dw_{after} being the weight of the samples collected in the field after 48 h.

The present study investigates the beetles' distribution among dung types as trophic networks and their community composition; an analysis of land-use effects on the beetles' biomass and overall removal activity has been presented by Frank et al. (see (Frank et al. 2017b)) based on the same survey data.

To characterize the distribution of decomposition rates across different dung types, we computed Shannon's evenness $E_{\text{dung}} = H'_{\text{dung}} / N_{\text{dung}}$, where $H'_{\text{dung}} = \Sigma\, p_j \bullet \log(p_j)$; p_j is the proportion of removal of dung type j of the total dung removal recorded per site, and N_{dung} represents the number of dung types (i.e. five in the comprehensive, six in the intensive survey). $E_1 = 0$ for a single dung type attended, and we excluded all cases where no dung was removed (69 out of 150 forests, 96 out of 150 grasslands).

Land-use intensity

For land-use characterization in grasslands, we used the land-use intensity index "LUI" (Blüthgen et al. 2012). This index is based on grazing (*G*), i.e. the number of livestock units times the number of days grazing per ha and year, fertilization (*F*), kg nitrogen applied per ha and year, and the frequency of mowing (*M*) per year. All parameters (*G, F, M*) are evaluated on annual basis (interview with farmers). Furthermore, all factors per site *i* for a given year were standardized relative to the mean within each corresponding region (*R)*. To reduce the impact of outliers and achieve a more even distribution, a square root-transformation was applied to the LUI, which is thus described as follows:

$$LUI = \sqrt{\frac{G_i}{G_R} + \frac{F_i}{F_R} + \frac{M_i}{M_R}}$$

When using individual compounds, we log-transformed LUI index components (*G* and *F*) to avoid outliers in regressions.

The forest management intensity index "ForMI"(Kahl and Bauhus 2014) is based on three parameters as well: the ratio of harvested tree volume to the sum of standing, harvested and dead wood volume (I_{harv}; a value of 0 describes no tree harvest in the last 30-40 years, a value of 1 a clear-cut site); the volume proportion of tree species that are not part of the natural forest composition, estimated as proportion of wood volume of non-native tree species to the sum of wood volume of all tree species (I_{nonat}; a value of 0 is a stand composed of natural forest vegetation only, 1 means that the whole stand consists of non-native tree-species); and the proportion of dead wood volume showing signs of saw cuts to the total amount of dead wood volume (I_{dwcut}; a value of 0 describes that all dead wood is a result of natural tree death, 1 that all dead wood is originated from management activity). These three parameters are summarized as:

$$ForMI = I_{harv} + I_{nonat} + I_{dwcut}$$

As the shift in tree species composition described in I_{nonat} comprises mainly coniferous species (*Picea abies* and *Pinus sylvestris*), we focus on the proportion of conifers below. For more details regarding the indices see (Blüthgen et al. 2012) for LUI and (Kahl and Bauhus 2014) for ForMI.

Beetle diversity and trophic networks

Each trophic network defined in this study summarizes the species-specific dung preferences of beetles, i.e. the distribution of individuals across the six dung traps per site. We focused on three parameters of the networks per site:

(a) Shannon diversity of dung beetles (H'), with $H' = \Sigma\, p_i \bullet \log(p_i)$, where p_i is the proportion of dung beetle species i of the total individuals captured at the site. For graphics, the exponential form $e^{H'}$ was displayed (suggested by Ref.(Jost 2006)), but in the models the untransformed H' was used as it showed a normal distribution unlike $e^{H'}$.

(b) Interaction diversity (H_2), i.e. the Shannon diversity of the realized links as a measure of the complexity of the network (see (Blüthgen 2010)). It is defined as $H_2 = \Sigma\, p_{ij} \bullet \log(p_{ij})$, where p_{ij} represents the proportion of the individuals of beetle species i trapped by dung type j among all beetle individuals in the traps. Network complexity expressed by H_2 increases with beetle diversity and number of dung types attended per beetle species and in total.

(c) The standardized specialization index H_2' describes the level of complementary specialization of a bipartite network ranging from 0 (no specialization, all species use different resources in similar proportions) to 1 (highly specialized, species differ strongly in their preferred resources). It is based on the above interaction diversity H_2, but standardized against the minimum and maximum H_2 possible for the network with given number of species and fixed number of individuals per species and resource.

As the index is standardized against variation in total abundance and diversity, it does not automatically increase with higher numbers of individuals recorded unlike H' and H_2 (see (Blüthgen et al. 2006)). In our analyses, H' and H_2 were computed for all sites with at least one dung beetle caught in the trap. $H' = 0$ for all sites with a single beetle species. $H_2 = 0$ for a 1×1 'network' (total $n = 134$ forests and $n = 75$ grasslands with at least one dung beetle). Complementary specialization H_2' can only be computed if at least two beetle species occur on at least two dung types per network (2×2 matrices, fulfilled by $n = 98$ forest and $n = 53$ grassland plots). For each species i, the standardized Kullback-Leibler distance d_i' (Blüthgen et al. 2006)describes its specialization in comparison to the choices of the other species, i.e. its exclusiveness. Again the index d_i' ranges from 0 (no specialization, same dung types preferred as other species) to 1 (highly specialized and exclusive). Networks were calculated per survey

per site (i.e. the momentary distribution of co-occurring species, representing their realized niches) or pooled across all forests and all grasslands for comparison (thus more closely reflecting the overall fundamental niches in different scenarios). For analyzing and visualizing, network analysis and graphics were conducted with the package 'bipartite' (Dormann et al. 2009).

Species-Specific Niche

To analyze the response of each dung beetle species to variation in land-use intensity, we calculated the abundance-weighted means (μ_i) of land-use intensity for each species, reflecting their 'optimum' along an environmental niche dimension (Chisté et al. 2016). The μ_i of a species i is defined as

$$\mu_i = \sum_{s=1}^{150} \sqrt{(a_{i,s})} \cdot L_s \Big/ \sum_{s=1}^{150} \sqrt{(a_{i,s})}$$

where s is each forest or grassland site, $a_{i,s}$ is the abundance of species i on site s, and L_s is the land-use intensity at site s. Note that the $a_{i,s}$ is square-root transformed to attenuate the weight of some sites in which a species occasionally occur with hundreds of individuals. We compared this μ_i with a null model allowing the species to occur in each site with the same probability, fixing the total number of sites in which the species occurs (10.000 randomizations performed). The proportion of μ_{null} smaller or larger than the observed μ_i provides the significance of each species land-use response. Species with observed values being significantly smaller than expected by the null model were defined as "loser species", species with significantly higher μ_i than expected as "winner species" (Chisté et al. 2016; code is available here: DOI: 10.13140/RG.2.2.13339.46881). The abundance-weighted standard deviation (σ_i) is also reported as a measure of niche breadth.

Data analysis

Mixed models (ANOVA, Type III) were employed to assess the contribution of (1) beetle diversity (H'), (2) network complexity (H_2) and (3) network specialization (H_2') to dung decomposition after controlling for temperature, region, habitat and total beetle biomass (Tab. S2, as we found no significant effects for temperature, we excluded this term from the models). Since the three focal predictors showed collinearity (Fig. S3), they were employed in separate models. The following three response variables were tested:

(1) the total dung decomposition rate, i.e. the sum of the burial of five dung types per site within 48h,

(2) the evenness of the dung removal across the five dung types H'/log(5) for the comprehensive, *H'*/log(6) for the intensive survey), with H' describing the Shannon diversity of decomposition rates, and

(3) the temporal fluctuation of the decomposition across the four months covered by the intensive surveys on 54 sites, expressed as the coefficient of variation (CV) of the total dung removal.

The mixed models for (1) and (2) were mainly computed for the comprehensive survey (based on decomposition, biomass, diversity and network indices computed for summer 2014 only). Analyses were repeated for the intensive survey based on mean decomposition rate, mean removal evenness, mean community biomass and mean beetle diversity across the four surveys; here, the two network indices H2 and H2' were pooled across the four survey months per site.

3.4 **Results**

Dung beetle abundance and diversity

In total we collected 18,770 beetle individuals from 34 species at the six dung types. Most beetle species showed pronounced habitat preferences or exclusively occurred in either forests or grasslands. The total dung beetle abundance was 2 – 11 times higher in forests than in grasslands in each of the three regions, and was associated with a higher diversity and increased overall dung decomposition rate of 2 – 6 times (Table 3.1, Extended Data Table S3.1 and Fig. S3.1).

Table 3.1. Properties of dung – beetle networks in 150 forests and 150 grasslands (mean ± sd). Statistical comparison between the habitats based on Mann-Whitney U-tests. Data for each region and for the intensive survey provided in Extended Data Table S3.2.

	Forests	Grasslands	U test
Beetle abundance	52.31 ± 84.65	3.0 ± 7.81	$U = 19380$, $P < 0.0001$
Beetle diversity H'	1.37 ± 0.70, $n = 134$*	0.8 ± 0.97, $n = 75$*	$U = 15940$, $P < 0.0001$
Network complexity H_2	1.35 ± 0.53, $n = 134$*	0.52 ± 0.57, $n = 75$*	$U = 8459$, $P < 0001$
Specialization H_2'	0.33 ± 0.29, $n = 98$*	0.50 ± 0.40, $n = 53$*	$U = 2055$, $P = 0.03$
Dung removal	31.56 ± 15.79	9.15 ± 9.48	$U = 2756$, $P < 0.0001$
Dung removal evenness E	0.64 ± 0.23, $n = 81$*	0.45 ± 0.30, $n = 54$*	$U = 8032.5$, $P < 0.0001$

*The computation of H' and H_2 requires at least one dung beetle species, H_2' at least two dung species and visitation of at least two dung types, and E also requires removal of at least two dung types

Dung-beetle networks in forests were more complex (increased H_2) and less specialized (decreased H_2') than those in grasslands (Table 1, Extended Data section, see Supplementary Information for definition and relationship of $H_2'$and H_2). The pooled data across sites showed a higher complexity and generalization than those per site, but showed the same distinction between forest and grassland (Fig. 3.1). These effects were independent of beetle biomass which was included in the models (Tables 1, Extended Data Table S3.2), and results were consistent for the comprehensive survey (Fig. 3.2a & b; Table 3.1) and the intensive survey (Fig. 3.2c, Extended Data Fig. S3.2, Table S3.2). Moreover, complexity (but not H_2') contributed to a more continuous dung removal over time (Fig. 3.2c, Table S3.2).

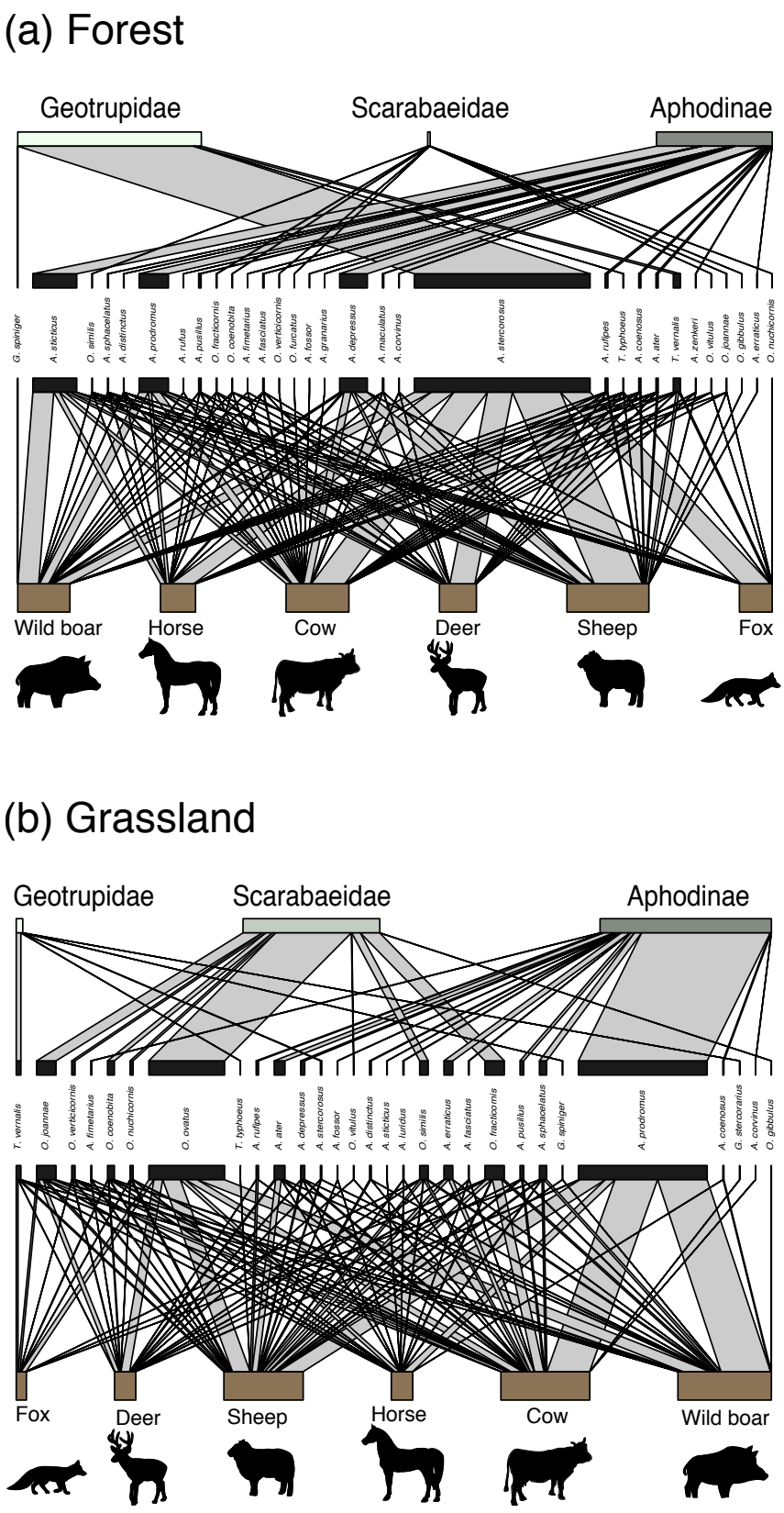


Figure 3.1: Networks show the distribution of dung beetle species across dung types, aggregated across 150 forests (a) and 150 grasslands (b). The upper level summarize the beetle species' higher taxa, which vary strongly in dominance across habitats. The networks show a low degree of complementary specialization at species level in forests (H_2' = 0.11) and grasslands (H_2' = 0.12).

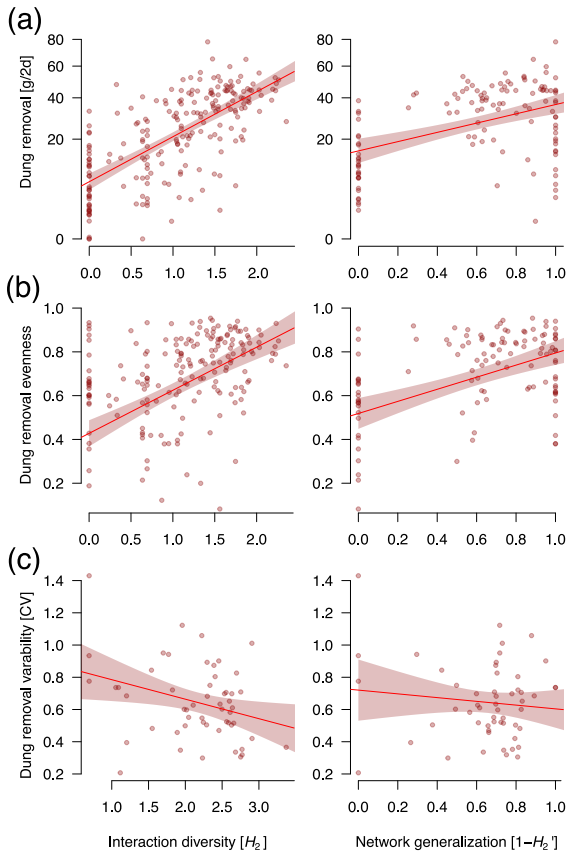


Figure 3.2: Effects of network properties on the performance and stability of an ecosystem service. For the comprehensive survey (n = 300 sites) (a) the overall rate of dung removal, (b) the evenness of removal rates across six dung types and for the intensive survey (n = 54 sites) (c) the temporal stability increased with the complexity (interaction diversity H_2) and generalization (1 – H_2') of dung – beetle networks. Stability is defined as the inverse of the variation (CV) over time (Tilman et al. 2006).

While dung decomposition showed some trends along management gradients (Frank et al. 2017b), the relatively stable dung communities and networks largely maintained this ecosystem function at a similar level, except for sites where too few beetles have been recorded (Fig. 3.3)

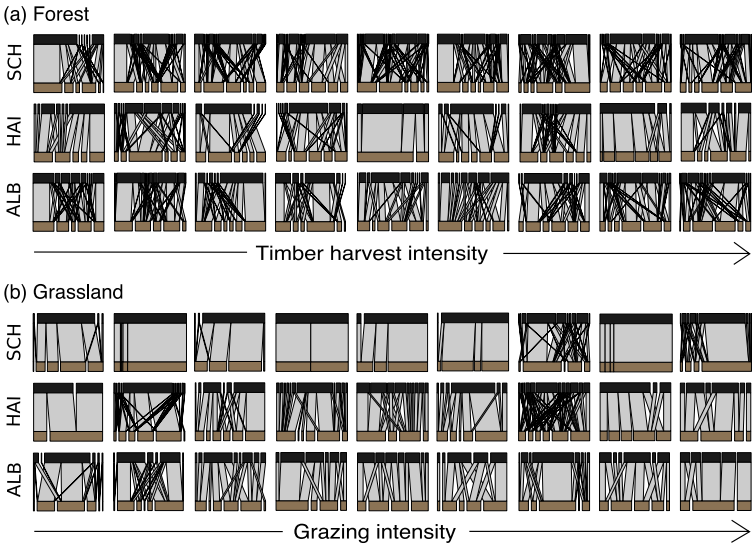


Figure 3.3: Trophic interaction networks per site in forests (a) and grasslands (b), represented by n = 9 sites per region and habitat that were surveyed more intensively. Networks show the sum of all individuals sampled and their distribution across dung types in different months in the Schorfheide (SCH), Hainich (HAI) and Alb (ALB), sorted by grazing intensity (lowest: ungrazed – highest: 457.4 life stock units*day/ha^{-1}y^{-1}) and timber-harvest intensity (lowest: no harvest – highest: 0.88 % of the timber volume harvested).

Species-specific responses

Comprehensive survey: Seven beetle species out of the total 22 species that occurred in forests (7/22 species) were significantly less abundant in sites with higher timber harvest intensity, whereas eight beetle species profited from the density of non-native tree species. A relatively low number of "losers" among the dung beetles was found for fertilization (3/17 species) and mowing (2/17 species) in grasslands. Since single components of land use contrast in their effects (e.g. negative effects of timber harvest, fertilization and mowing, but positive responses for the proportion of non-native tree species and grazing, Table 3.2, 3.3), net effects of compound land-use intensity gradients (LUI and ForMI) appear neutral (forest: 18/22 species, grassland: 14/17 species) (Fig. 3.4a, b).

Intensive survey: in forests, 3/26 species were "losers" of ForMI, while in grasslands 2/27 species were "winners" of LUI (Fig. 3.4c, d). Again, the vast majority of species remained unaffected while single components in forests and grasslands non-significantly had negative (timber harvest: 3/26 species) or positive (fertilization: 5/26 species, mowing 4/26 species) effects on the abundance of single species (Table S3.5, S3.6).

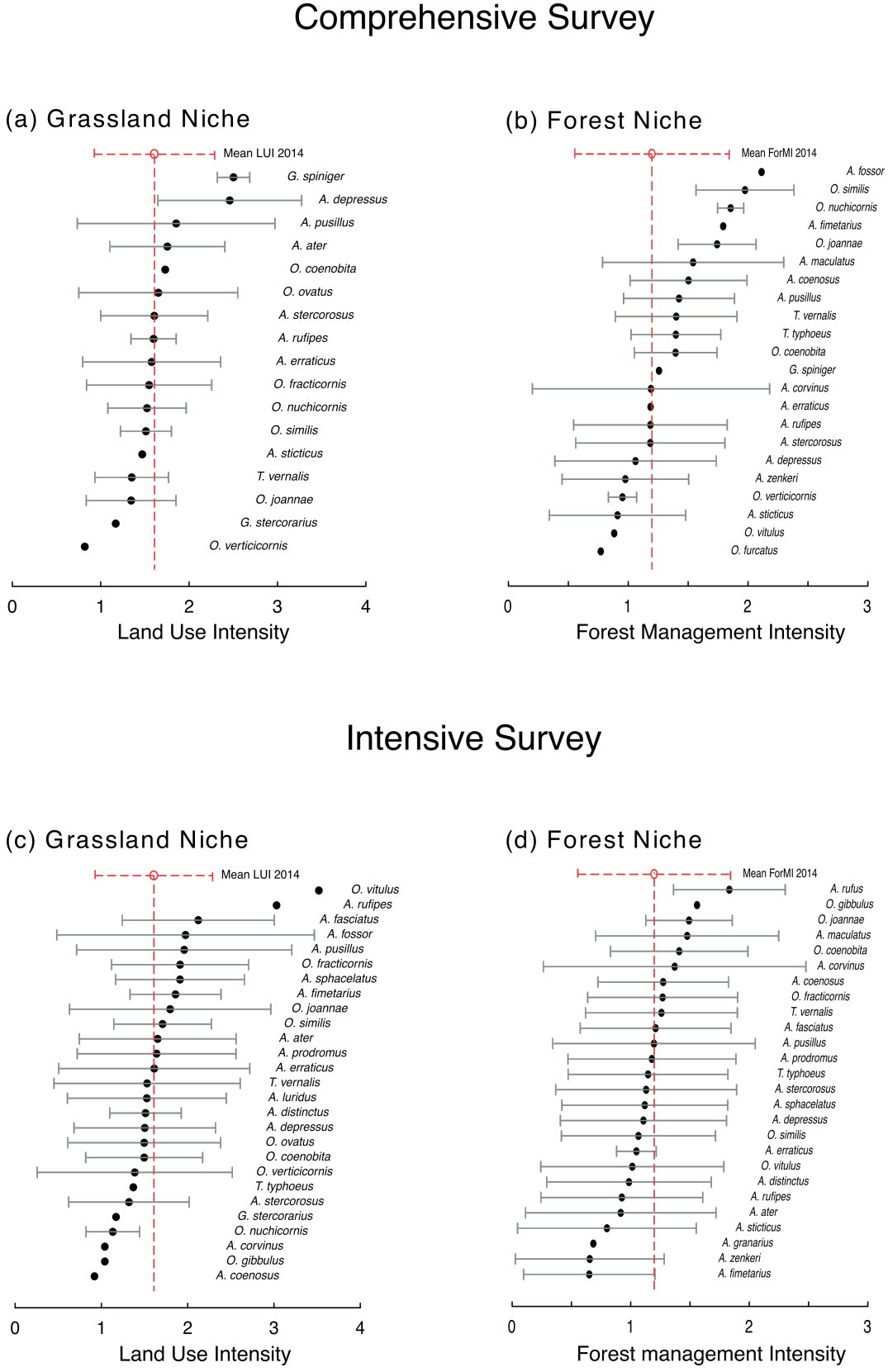


Figure 3.4: Land-use niches (abundance-weighted mean and standard deviation; $\mu_i \pm \sigma_i$) of the habitat-specific land-use indices for each dung beetle species sampled during the comprehensive survey in grassland (a) and forest (b), and intensive survey in grassland (c) and forest (d). Dashed lines represent the mean land-use levels and standard deviation (SD) across all 150 sites per habitat. Species with μ_i to the right side of the dashed line are termed "winner", to the left of the dashed line as "loser".

Table 3.2: Land-use niches for all dung beetle species caught during the comprehensive survey in forests. The table shows for each species the number of sites where it occurs (*N*) and the abundance-weighted mean with abundance-weighted standard deviation ($\mu_i \pm \sigma_i$) for the forest management intensity index and its components. Corresponding *P*-values highlight the deviance of each species towards the mean land-use level, which results in species-specific fate.

		Forest Management Intensity			Timber harvest			Non-native tree species			Dead wood with saw cuts		
Species	*N*	$\mu_i \pm \sigma_i$	*P*-value	Fate	$\mu_i \pm \sigma_i$	*P*-value	Fate	$\mu_i \pm \sigma_i$	*P*-value	Fate	$\mu_i \pm \sigma_i$	*P*-value	Fate
Anoplotrupes stercorosus	126	1.18 ± 0.62	0.269	neutral	**0.29 ± 0.21**	**0**	**loser**	**0.29 ± 0.41**	**0.046**	**winner**	0.6 ± 0.26	0.204	neutral
Aphodius coenosus	7	1.5 ± 0.49	0.093	neutral	**0.18 ± 0.07**	**0.024**	**loser**	**0.71 ± 0.46**	**0.003**	**winner**	0.55 ± 0.21	0.327	neutral
Aphodius corvinus	3	1.19 ± 0.99	0.491	neutral	**0.11 ± 0.08**	**0.022**	**loser**	0.52 ± 0.5	0.136	neutral	0.56 ± 0.46	0.375	neutral
Aphodius depressus	39	1.06 ± 0.67	0.066	neutral	**0.28 ± 0.19**	**0.037**	**loser**	0.24 ± 0.38	0.312	neutral	0.59 ± 0.27	0.424	neutral
Aphodius erraticus	1	1.19 ± NA	0.432	neutral	0.34 ± NA	0.406	neutral	0.09 ± NA	0.338	neutral	0.76 ± NA	0.328	neutral
Aphodius fimetarius	1	1.79 ± NA	0.196	neutral	0.31 ± NA	0.47	neutral	0.55 ± NA	0.255	neutral	**0.93 ± NA**	**0.044**	**winner**
Aphodius fossor	1	2.11 ± NA	0.115	neutral	0.23 ± NA	0.351	neutral	**1 ± NA**	**0.016**	**winner**	0.88 ± NA	0.068	neutral
Aphodius maculatus	18	**1.54 ± 0.76**	**0.005**	**winner**	0.44 ± 0.32	0.028	winner	0.34 ± 0.47	0.176	neutral	0.67 ± 0.25	0.122	neutral
Aphodius pusillus	3	1.42 ± 0.46	0.246	neutral	0.27 ± 0.15	0.346	neutral	0.39 ± 0.33	0.205	neutral	0.74 ± 0.08	0.17	neutral
Aphodius rufipes	22	1.19 ± 0.64	0.489	neutral	0.34 ± 0.2	0.42	neutral	0.27 ± 0.4	0.467	neutral	0.6 ± 0.2	0.475	neutral
Aphodius sticticus	28	**0.91 ± 0.57**	**0.004**	**loser**	**0.23 ± 0.18**	**0.003**	**loser**	0.2 ± 0.33	0.197	neutral	0.57 ± 0.31	0.258	neutral
Aphodius zenkeri	6	0.98 ± 0.53	0.197	neutral	0.38 ± 0.2	0.32	neutral	0.14 ± 0.37	0.197	neutral	0.54 ± 0.25	0.287	neutral
Geotrupes spiniger	1	1.26 ± NA	0.382	neutral	0.32 ± NA	0.44	neutral	0 ± NA	0.444	neutral	**0.94 ± NA**	**0.025**	**winner**
Onthophagus coenobita	7	1.4 ± 0.34	0.205	neutral	**0.2 ± 0.15**	**0.04**	**loser**	**0.58 ± 0.43**	**0.016**	**winner**	0.62 ± 0.24	0.447	neutral
Onthophagus furcatus	1	0.77 ± NA	0.258	neutral	0.15 ± NA	0.23	neutral	0 ± NA	0.464	neutral	0.62 ± NA	0.434	neutral
Onthophagus joannae	6	**1.74 ± 0.33**	**0.018**	**winner**	0.24 ± 0.14	0.168	neutral	**0.76 ± 0.38**	**0.004**	**winner**	0.71 ± 0.11	0.149	neutral
Onthophagus nuchicornis	2	1.85 ± 0.11	0.09	neutral	0.26 ± 0.24	0.375	neutral	**0.79 ± 0.3**	**0.045**	**winner**	0.78 ± 0.05	0.194	neutral
Onthophagus similis	2	1.97 ± 0.41	0.055	neutral	0.27 ± 0.2	0.398	neutral	**0.99 ± 0.02**	**0.01**	**winner**	0.72 ± 0.2	0.315	neutral
Onthophagus verticicornis	2	0.95 ± 0.12	0.296	neutral	0.22 ± 0.02	0.238	neutral	0 ± 0	0.266	neutral	0.74 ± 0.1	0.248	neutral
Onthophagus vitulus	1	0.88 ± NA	0.326	neutral	0.21 ± NA	0.322	neutral	0 ± NA	0.486	neutral	0.67 ± NA	0.49	neutral
Trypocopris vernalis	36	**1.4 ± 0.51**	**0.018**	**winner**	**0.22 ± 0.13**	**0**	**loser**	**0.46 ± 0.44**	**0**	**winner**	0.64 ± 0.24	0.162	neutral
Typhaeus typhoeus	3	1.4 ± 0.37	0.281	neutral	0.31 ± 0.25	0.483	neutral	0.33 ± 0.56	0.35	neutral	0.75 ± 0.09	0.159	neutral

Table 3.3: Land-use niches for all dung beetle species caught during the comprehensive survey in grasslands. The table shows for each species the number of sites where it occurs (*N*) and the abundance-weighted mean with abundance-weighted standard deviation ($\mu_i \pm \sigma_i$) for the land-use intensity index and its components. Corresponding *P*-values highlight the deviance of each species towards the mean land-use level, which results in species-specific fate.

		Land-Use Intensity			log Grazing			log Fertilization			Mowing		
Species	*N*	$\mu_i \pm \sigma_i$	*P*-value	Fate	$\mu_i \pm \sigma_i$	*P*-value	Fate	$\mu_i \pm \sigma_i$	*P*-value	Fate	$\mu_i \pm \sigma_i$	*P*-value	Fate
Anoplotrupes stercorosus	5	1.61 ± 0.61	0.484	neutral	**15.25 ± 6.11**	**0.026**	**winner**	2.39 ± 3.5	0.453	neutral	**0.2 ± 0.44**	**0.004**	**loser**
Aphodius ater	5	1.75 ± 0.65	0.291	neutral	7.44 ± 4.44	0.355	neutral	4.66 ± 4.88	0.176	neutral	1.76 ± 1.29	0.105	neutral
Aphodius depressus	3	**2.46 ± 0.81**	**0.019**	**winner**	**1.53 ± 3.15**	**0.031**	**loser**	**8.16 ± 4.27**	**0.027**	**winner**	**2.36 ± 0.59**	**0.011**	**winner**
Aphodius erraticus	12	1.58 ± 0.78	0.46	neutral	11.51 ± 8.67	0.064	neutral	3 ± 3.73	0.438	neutral	1.02 ± 1.29	0.403	neutral
Aphodius pusillus	4	1.85 ± 1.12	0.216	neutral	7.68 ± 5.16	0.435	neutral	5.42 ± 7.82	0.121	neutral	1.45 ± 1.28	0.299	neutral
Aphodius rufipes	6	1.6 ± 0.26	0.491	neutral	8.06 ± 8.79	0.479	neutral	2.81 ± 3.31	0.492	neutral	1.27 ± 1.21	0.355	neutral
Aphodius sticticus	1	1.47 ± NA	0.467	neutral	16.35 ± NA	0.136	neutral	0 ± NA	0.33	neutral	0 ± NA	0.347	neutral
Geotrupes spiniger	2	**2.5 ± 0.18**	**0.045**	**winner**	0 ± 0	0.086	neutral	**9.51 ± 1.24**	**0.03**	**winner**	**3 ± 0**	**0.019**	**winner**
Geotrupes stercorarius	1	1.17 ± NA	0.279	neutral	7.62 ± NA	0.462	neutral	0 ± NA	0.351	neutral	1 ± NA	0.34	neutral
Onthophagus coenobita	1	1.73 ± NA	0.343	neutral	15.07 ± NA	0.172	neutral	5.41 ± NA	0.316	neutral	0 ± NA	0.355	neutral
Onthophagus fracticornis	19	1.55 ± 0.71	0.354	neutral	8.94 ± 6.41	0.35	neutral	1.71 ± 4.2	0.097	neutral	0.92 ± 1.05	0.202	neutral
Onthophagus joannae	19	**1.34 ± 0.51**	**0.024**	**loser**	10.25 ± 5.52	0.11	neutral	1.55 ± 3.21	0.079	neutral	0.8 ± 1.1	0.098	neutral
Onthophagus nuchicornis	9	1.52 ± 0.44	0.356	neutral	9.87 ± 9.8	0.241	neutral	**0.64 ± 1.74**	**0.036**	**loser**	1.08 ± 0.79	0.45	neutral
Onthophagus ovatus	20	1.65 ± 0.9	0.369	neutral	**11.6 ± 10.62**	**0.017**	**winner**	2.88 ± 3.99	0.497	neutral	1.14 ± 1.07	0.465	neutral
Onthophagus similis	11	1.51 ± 0.29	0.321	neutral	11.33 ± 7.54	0.094	neutral	**0.23 ± 0.82**	**0.01**	**loser**	0.93 ± 0.95	0.303	neutral
Onthophagus verticicornis	1	0.82 ± NA	0.081	neutral	9.12 ± NA	0.452	neutral	0 ± NA	0.335	neutral	0 ± NA	0.343	neutral
Trypocopris vernalis	13	1.35 ± 0.42	0.071	neutral	11.58 ± 6.4	0.055	neutral	**0.7 ± 1.84**	**0.015**	**loser**	**0.54 ± 0.66**	**0.01**	**loser**

3.5 **Discussion**

Dung beetle communities in forests substantially differed from those in grasslands: their abundance and diversity in forests was much higher, resulting in a higher dung-removal rate (see also Frank et al. 2017b). The species composition also showed a strong differentiation, e.g. most larger Geotrupidae and many Aphodius occurred primarily in forests, several Onthophagus in grasslands (Table S3.1). Such a differentiation between forests and grasslands is consistent with dung beetle communities in the tropics (Nummelin and Hanski 1989, Davis et al. 2001), where forest removal is often associated with strong declines in diversity and abundance (Howden and Nealis 1975, Hanski 1989, Klein 1989, Estrada et al. 1999). The changes in communities from forests to grasslands in our study may partly mirror the historical impact of habitat conversion from forests to open secondary vegetation that have shaped central the European cultural landscape over the last centuries.

Although vertebrate feces varies in volatile organic compounds and nutrient composition, with systematic differences between herbivorous, omnivorous, and carnivorous dung producers (Frank et al. 2017a), dung beetles have been described to be relatively generalized in their dung type choices, regardless on which dung they have been raised on (Hanski and Cambefort 1991, Dormont et al. 2010). Network analysis confirmed quantitatively that beetle species were highly generalized and relatively similar in their choices of dung types, although choices were significantly non-random compared to null models (see Extended Data section). Dung-beetle networks in forests were more complex (increased H_2) and less specialized (decreased H_2') than those in grasslands (Table 3.1, Extended Data section, see Supplementary Information for definition and relationship of H_2'and H_2). The pooled data across sites showed a higher complexity and generalization than those per site, but showed the same distinction between forest and grassland (Fig. 3.1). These effects were independent of beetle biomass which was included in the models (Tables 3.1, Extended Data Table S3.2), and results were consistent for the comprehensive survey (Fig. 3.2a & b; Table 3.1) and the intensive survey (Fig. 3.2c, Extended Data Fig. S3.2, Table S3.2). Moreover, complexity (but not H_2') contributed to a more continuous dung removal over time (Fig. 3.2c, Table S3.2). Interestingly, the beetles' species diversity (*H'*) alone, although an important determinant of network complexity (Extended Data Fig. S3.3), did not significantly improve dung decomposition, underscoring the functional importance of such networks(Tylianakis et al. 2007).

Land use, such as forest habitat conversion and agriculture, is the main driver of current biodiversity losses (Sala et al. 2000). As a result, affected species communities show a loss of ecosystem services, such as nutrient cycling or protection against soil erosion (Nichols et al. 2007, Hautier et al. 2015). Negative land-use effects on dung beetle communities were reported in grasslands and tropical forests where fundamental habitat disturbances were involved (Nichols et al. 2007). In our study, the substantial decline in dung beetle abundance and decomposition rates from forests to grasslands is driven by the absence of very few large geotrupid species in open habitats (Extended Data Fig. S3.4). Similarly to reports from other studies(Larsen et al. 2005, Gardner et al. 2008), this is a marked example of the higher vulnerability of larger dung beetle species to disturbance or fragmentation. More rapid losses of large species results in a stronger functional decline in ecosystems than predicted by random extinction order(Larsen et al. 2005). Within forests and grasslands variation in land-use intensity had only weak effects on total abundance and diversity of dung beetles (Extended Data Table S3.4, Fig. S3.5). Moreover, dung – beetle networks did not show a significant change in H_2 and H_2' along most management gradients within forests and within grasslands, although a decrease in network complexity with the amount of timber harvested was found. This suggests that timber extraction has the strongest overall impact apart from forest conversion to open grasslands. While dung decomposition showed some trends along management gradients (Frank et al. 2017b), the relatively stable dung communities and networks largely maintained this ecosystem function at a similar level, except for sites where too few beetles have been recorded (Fig. 3.3).

Species-specific responses

The individual species' land-use responses (i.e. their abundance-weighted mean of land-use intensity on which the populations occurred) were more differentiated. Corresponding to these weak effects at the community level, our analysis of responses by individual species (their land-use niche) confirmed that a majority of species were relatively unaffected by land-use intensity within a habitat. Yet, for timber harvest intensity, the highest proportion of significant "losers" was found (32 % of the species in forests). In grasslands three species (18%) appeared as "losers" of fertilization intensity (only two species suffered from mowing and only a single species from grazing and overall LUI). The proportion of losers in grassland was relatively low compared to other taxa recorded in the same sites such as grasshoppers (52% losers of LUI, Chisté et al. 2016), cicadas (34%, Chisté et al., submitted), moths (27%, Mangels et al., submitted) and bumblebees (25%, Kämper et al.

(2016)). Since land-use intensity negatively affects diversity in many taxa (Allan et al. 2014, Allan et al. 2015, Newbold et al. 2015, Soliveres et al. 2016), we had expected similar effects in dung beetle communities. Indeed, strong negative land-use effects were reported elsewhere in grasslands and tropical forests (Hutton and Giller 2003, Nichols et al. 2007, Barragan et al. 2011), comprising more fundamental habitat disturbances compared to the intensity gradients studied here. In forests, management such as massive timber harvest only occurs once in several years, allowing the beetle populations to recover as long as the areas are large and/or connected to relatively undisturbed forests. Hence, heavy disturbances such as gaps created by previous timber harvest did show negative, albeit not significant trends for total abundance and diversity of dung beetles in our study. In contrast, grasslands are managed on an annual basis, and dung beetles are faced with constant changes and disturbance of their habitat or need to be more mobile and use the surrounding habitats. In grasslands single land-use components showed differing effects, still the patterns stay consistent: contrary effects result in neutral fates, while single or accumulating effects discriminate or benefit certain species (Table 3.2 & 3.3). Mixed management strategies applied together, or combinations of different management strategies in compound land-use gradients such as LUI or ForMI may then yield an overall impression of neutral responses for most of the species. In fact, the most abundant species (*A. stercorosus, A. depressus, A. prodromus, O. fracticornis, O. ovatus*), representing 78 % of all collected specimen, showed responses towards single components, but not for compound LUI or ForMI.

Conclusion

We were able to show effects of habitat management on single dung beetle species, as several species either suffered (seven species declined by timber harvest) or profited from land-use intensity (eight species increased with rising proportion of conifers), highlighting both, positive and negative land-use effects. These results highlight the importance of generalized functional networks for the stability of an important ecosystem function – dung decomposition – against land-use intensity impacts within forest and grassland habitats. Higher dung beetle abundance and diversity improved the trophic network complexity, and a higher generalization of dung use among the co-existing species contributed to higher – independently measured – decomposition rates and more balanced removal of different dung types (Fig. 3.1, Extended Data Fig. S3.6 & S3.7). We thus demonstrate that a relevant ecosystem function not only depends on the diversity of species (which showed no effect *per se*), but most importantly on the complexity of the networks formed by different functional

performers. However, significant negative effects were found at the species level. Such subtle effects beyond the total diversity and abundance confirm the value of in-depth analyses of species interactions (networks) as well as single species responses. Niche models based on the abundance-weighed mean of environmental gradients (here: land-use intensity) provide a suitable tool for examining consequences at a species level (Chisté et al. 2016).

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3.6 Supplementary Material to Chapter 3

Extended Data and Results

Dung beetle abundance and diversity

Dung beetle communities in forests substantially differed from those in grasslands: their abundance and diversity in forests was much higher, resulting in a higher dung-removal rate (see also Frank et al. 2017b) (Table 3.1, Fig. 3.1).

Larger Geotrupidae species such as *Anoplotrupes stercorosus* and *Trypocopris vernalis* were most abundant in forests, whereas *Onthophagus* species such as *Onthophagus fracticornis*, *O. similis* and *O. joannae* predominantly occurred in grasslands. *Aphodius* species show distinct (*Aphodius depressus* and *A. erraticus*) or intermediate preferences for a habitat (*Aphodius ater* and *A. sphacelatus*). Eight species were exclusively found in either forest or grasslands (e.g. *Onthophagus ovatus, Aphodius luridus, A. rufus, A. zenkeri*) (Table S1). Such a differentiation between forests and grasslands is consistent with dung beetle communities in the tropics (Nummelin and Hanski 1989, Davis et al. 2001), where forest removal is often associated with strong declines in diversity and abundance (Howden and Nealis 1975, Hanski 1989, Klein 1989, Estrada et al. 1999). Dung beetle densities were two times higher in forests than in grasslands in the Hainich ($t = 9.19$, $P < 0.001$), four times in the Alb (Welch t-test, $t = 9.41$, $P < 0.001$), and eleven times in the Schorfheide ($t = 26.25$, $P < 0.001$) (Table S3.1, Fig. S3.1).

Comprehensive survey: in forests, beetle abundance did not change significantly with management intensity, and species richness was mostly unaffected except for a significant increase with the proportion of non-native tree species (conifers) (Table S3.4). In grassland sites, the beetles' abundance faced contrary effects of different management types. While grazing intensity was associated with a significant increase in total abundance (by +65 % along the gradient), fertilization and mowing led to a significant decrease in abundance by –30% and –53%, respectively (Table S3.4).

Dung beetle richness S and Shannon diversity H' were higher in forests than in grasslands (mean ± sd; forests: $S = 2.14 \pm 1.44$; $H' = 0.33 \pm 0.33$; grasslands: $S = 0.88 \pm 1.11$; $H' = 0.19 \pm 0.35$), and the difference was significant (Mann-Whitney U tests: $U_S = 17228$, $P_S < 0.001$; $U_{H'} = 14900$, $P_{H'} < 0.001$). However, beetle diversity was unaffected by variation in land-use intensity within forests or grasslands (Table S3.4).

Intensive survey: neither total abundance, nor richness *S* and diversity *H'* showed a significant response to land-use intensity (Table S3.4). Again, *S* and *H'* were higher in forests than in grasslands (forests: $S = 3.77 \pm 2.53$; $H' = 0.64 \pm 0.47$; grasslands: $S = 1.94 \pm 2.34$; $H' = 0.39 \pm 0.54$; $U_S = 8618.5$, $P_S < 0.001$; $U_{H'} = 7755.5$, $P_{H'} < 0.001$).

Our sample of 50 sites per habitat and region for the comprehensive survey represented the regional dung beetle species pool very well: the dung beetle richness in forests showed ≥ 87.5 % sampling completeness when the recorded and estimated richness (Chao1) were compared in each region, and for grasslands the estimated completeness was even 100 %. The same is true for the 36 sites per habitat and region for the intensive surveys, as the richness in forests showed ≥85.7 % completeness, while the grasslands completeness was ≥ 86.9 %.

Dung–beetle networks

Overall, dung beetles preferred some dung types over others. In dung-baited traps, cow dung attracted the highest, horse dung the lowest number of beetles (see brown bar widths of dung types in Fig. 3.1). Differences across dung types were significant (forest: $F_{5, 1374} = 3.8$, $P < 0.0001$; grassland: $F_{5, 1380} = 5.3$, $P < 0.0001$). Consequently, dung removal rates were also variable across dung types (forest: $F_{5, 1044} = 22.2$, $P < 0.0001$; grassland: $F_{5, 1003} = 17.5$, $P < 0.0001$). In forests, cow and wild boar dung were the most attractive dung types for the beetles, followed by sheep, deer and horse dung. The pattern in grasslands was similar (Fig. S3.1). The partitioning of dung types among beetle species (specialization H_2') in each site was higher than for the pooled network across sites (forests: $H_2' = 0.11$, grasslands: $H_2' = 0.12$, Fig. 3.1). Interaction diversity (H_2) did not change consistently along the land-use gradients , except for single components showing a significant increase with the proportion of conifers (extensive survey) and a decrease with fertilization (intensive survey) (Fig. S3.4a, d & e). The beetles diversity (*H*') showed no effects for management, except for a significant decrease with fertilization (extensive survey) (Fig. S3.4c). H_2' was also similar along the gradients, except for significant decrease with the proportion of conifers (extensive survey) and grazing (intensive survey) (Fig. S3.4b & f). Dung types were also attended more evenly in forests than in grasslands (Table 3.1, Table S3.1).Across all sites, a higher trophic network complexity (H_2) was related to beetle abundance and diversity, but also higher resource overlap of the co-existing dung beetles in each community reflected by H_2' which quantifies specialization independent of variation in species' abundance and diversity (Fig. S3.7a).

Table S3.1: List of dung beetle species with number of individuals collected for each region (SCH, HAI, ALB) and habitat (forest = F, grassland = G). Habitat preference was tested with χ^2-analysis. *P*-values show significance of habitat preference. No habitat preference is marked with ´NA´.

Region	Schorfheide		Hainich		Alb		Total individuals	χ^2	*P*-value	Habitat preference
Habitat	F	G	F	G	F	G				
Anoplotrupes	6847	6	1350	0	1126	6	9335	9287.1	< 0.0001	Forest
Trypocopris vernalis	379	17	0	2	0	23	421	269.8	< 0.0001	Forest
Typhaeus typhoeus	38	1	0	0	0	0	39	35.1	< 0.0001	Forest
Geotrupes spiniger	0	0	2	0	0	3	5	0.2	0.65	NA
Geotrupes	0	0	0	0	0	1	1	1	0.32	NA
Onthophagus	12	7	4	42	5	13	83	20.3	< 0.0001	Grassland
Onthophagus	32	44	0	6	13	125	220	76.1	< 0.0001	Grassland
Onthophagus furcatus	2	0	0	0	0	0	2	2	0.16	NA
Onthophagus gibbulus	0	0	0	1	1	0	2	0	1	NA
Onthophagus joannae	23	2	0	54	1	119	199	114.6	< 0.0001	Grassland
Onthophagus	3	27	0	0	0	0	30	19.2	< 0.0001	Grassland
Onthophagus ovatus	0	14	0	670	0	0	684	684	< 0.0001	Grassland
Onthophagus similis	25	49	0	3	6	25	108	19.6	< 0.0001	Grassland
Onthophagus vitulus	0	0	1	0	2	4	7	0.1	0.71	NA
Onthophagus	0	0	3	28	0	2	33	22.1	< 0.0001	Grassland
Aphodius ater	32	21	10	74	75	2	214	1.9	0.17	NA
Aphodius coenosus	79	0	1	9	32	0	121	87.7	< 0.0001	Forest
Aphodius corvinus	2	0	2	1	19	0	24	20.2	< 0.0001	Forest
Aphodius depressus	1145	2	121	12	202	22	1504	1363.5	< 0.0001	Forest
Aphodius distinctus	26	14	1	1	0	0	42	3.4	0.06	NA
Aphodius erraticus	0	5	2	63	1	12	83	71.4	< 0.0001	Grassland
Aphodius fasciatus	62	0	6	6	0	0	74	51.9	< 0.0001	Forest
Aphodius fimetarius	2	4	3	0	1	5	15	0.6	0.44	NA
Aphodius fossor	1	0	0	2	0	0	3	0.3	0.56	NA
Aphodius granarius	1	0	0	0	0	0	1	1	0.32	NA
Aphodius luridus	0	0	0	6	0	3	9	9	0.003	Grassland
Aphodius maculatus	0	0	53	0	17	0	70	70	< 0.0001	Forest
Aphodius prodromus	763	810	281	124	510	228	2716	56.6	< 0.0001	Forest
Aphodius pusillus	59	3	1	28	68	2	161	56.1	< 0.0001	Forest
Aphodius rufipes	30	0	33	9	82	12	166	92.6	< 0.0001	Forest
Aphodius rufus	3	0	0	0	1	0	4	4	0.05	Forest
Aphodius sphacelatus	27	10	18	42	5	13	115	1.9	0.16	NA
Aphodius sticticus	2181	2	19	0	129	0	2331	2323	< 0.0001	Forest
Aphodius zenkeri	1	0	13	0	18	0	32	32	< 0.0001	Forest
Total individuals	1177	1038	1924	1183	2314	620	18854			

Table S3.2: ANOVA (Type III tests) for the response variables total dung removal (square-root transformed), evenness of dung removal and temporal fluctuation expressed as the coefficient of variation (CV) of the total dung removal. Sum of squares (SS), *F* values and significance level (*P*) are shown for three models, each with one of the alternative predictors Shannon Diversity (*H'*), network complexity (H_2) and network generalisation (H_2') (degrees of freedom: *df* = 1 for all factors, except 'region' where *df* = 2). (a) Comprehensive sampling contains n = 300 sites, (b) intensive sampling contains four surveys on n = 54 sites.

(a) Comprehensive survey

Response	Predictor	SS			*F*			*P*		
	Region	1.85	4.05	1.06	0.54	1.25	0.41	0.58	0.29	0.66
	Habitat	7.08	4.68	0.48	4.15	2.88	0.37	0.04	0.09	0.54
Removal	Beetle biomass[1)]	124.03	45.63	58.02	72.08	28.11	44.85	0.0001	0.0001	0.0001
	H	2.14	–	–	1.26	–	–	0.26	–	–
	H_2	–	18.51	–	–	11.40	–	–	0.001	–
	H_2'	–	–	6.37	–	–	4.92	–	–	0.02
	Region	0.32	0.26	0.07	3.15	2.57	1.06	0.04	0.07	0.35
	Habitat	0.02	0.01	0.03	0.40	0.16	0.93	0.53	0.69	0.34
Evenness	Beetle biomass[2)]	0.94	0.31	0.42	18.51	6.22	12.19	0.001	0.01	0.001
	H	0.07	–	–	1.39	–	–	0.24	–	–
	H_2	–	0.18	–	–	3.69	–	–	0.06	–
	H_2'	–	–	0.31	–	–	9.00	–	–	0.003

(b) Intensive survey

Response	Predictor	SS			*F*			*P*		
	Region	0.27	0.31	0.22	2.68	3.16	2.08	0.08	0.05	0.14
	Habitat	0.03	0.001	0.04	0.50	0.03	0.71	0.48	0.87	0.41
CV	Beetle biomass[3)]	0.04	0.01	0.03	0.81	0.22	0.65	0.37	0.64	0.42
	H	0.12	–	–	2.45	–	–	0.12	–	–
	H_2	–	0.22	–	–	4.53	–	–	0.04	–
	H_2'	–	–	0.01	–	–	0.20	–	–	0.65
	Region	0.79	1.81	0.71	0.75	1.88	0.70	0.48	0.16	0.50
	Habitat	0.41	0.36	0.77	0.77	0.76	1.50	0.38	0.39	0.23
Removal	Beetle biomass[4)]	15.53	9.17	12.02	29.42	19.13	23.29	0.0001	0.0001	0.0001
	H	1.56	–	–	2.97	–	–	0.09	–	–
	H_2	–	3.89	–	–	8.10	–	–	0.006	–
	H_2'	–	–	2.13	–	–	4.13	–	–	0.05
	Region	0.22	0.24	0.20	6.33	7.20	5.26	0.003	0.002	0.008
	Habitat	0.02	0.00	0.02	0.89	0.02	0.82	0.35	0.90	0.37
Evenness	Beetle biomass[5)]	0.17	0.12	0.24	9.92	7.63	12.82	0.003	0.01	0.001
	H	0.07	–	–	3.86	–	–	0.06	–	–
	H_2	–	0.11	–	–	6.79	–	–	0.01	–
	H_2'	–	–	0.003	–	–	0.17	–	–	0.69

1) $f(x) = 2.59 + 6.49(1-\exp(-x/1.04))$, where *x* is the total biomass of dung beetles
2) $f(x) = 0.42 + (0.83-0.42)/(1+(0.75/x))$, where *x* is the total biomass of dung beetles
3) total biomass as linear term
4) $f(x) = 2.94 + 5.91(1-\exp(-x/1.68))$, where *x* is the total biomass of dung beetles
5) $f(x) = (0.68)/(1+(0.006/x))$, where *x* is the total biomass of dung beetles

Table S3.3. Properties of dung – beetle networks during four surveys in 54 forests and 54 grasslands (mean ± sd) for each region (Schorfheide, Hainich and Alb). Statistical comparison between the habitats based on Mann-Whitney U-tests.

Region		Forests	Grasslands	U test
	Beetle abundance	181.23 ± 282.31	26.53 ± 56.49	$U = 1145$, $P < 0.0001$
	Beetle diversity H'	2.34 ± 0.84	0.75 ± 1.10	$U = 1148$, $P < 0.0001$
Schorfheide	Network complexity e^{H2}*	6.81 ± 2.32	3.18 ± 2.13	$U = 520$, $P < 0.0001$
	Specialization H_2'*	0.29 ± 0.16	0.57 ± 0.49	$U = 154$, $P = 0.45$
	Dung removal	38.68 ± 15.07	8.26 ± 7.07	$U = 1172$, $P < 0.0001$
	Dung removal evenness E	0.86 ± 0.13	0.70 ± 0.33	$U = 655$, $P = 0.09$
	Beetle abundance	31.71 ± 28.49	29.28 ± 70.56	$U = 986$, $P = 0.001$
	Beetle diversity H'	1.73 ± 0.81	1.87 ± 1.83	$U = 748$, $P = 0.48$
Hainich	Network complexity e^{H2}*	4.55 ± 1.75	4.92 ± 4.34	$U = 583$, P =0.39
	Specialization H_2'*	0.32 ± 0.30	0.3 ± 0.32	$U = 304$, $P = 0.62$
	Dung removal	23.50 ± 18.85	11.34 ± 7.47	$U = 809$, $P = 0.01$
	Dung removal evenness E	0.78 ± 0.26	0.75 ± 0.32	$U = 543$, $P = 0.89$
	Beetle abundance	50.81 ± 60.82	12.89 ± 23.75	$U = 988$, $P = 0.0001$
	Beetle diversity H'	2.15 ± 1.43	1.59 ± 1.44	$U = 780$, $P = 0.14$
Alb	Network complexity e^{H2}*	5.77 ±3.84	3.86 ± 2.75	$U = 535$, $P = 0.05$
	Specialization H_2'*	0.31 ± 0.26	0.53 ± 0.35	$U = 145$, $P = 0.05$
	Dung removal	23.30 ± 15.12	12.54 ± 10.47	$U = 789$, $P = 0.001$
	Dung removal evenness E	0.69 ± 0.33	0.65 ± 0.35	$U = 526$, $P = 0.45$

*The computation of H' and H_2 requires at least one dung beetle species, H_2' at least two dung species and visitation of at least two dung types, and E also requires removal of at least two dung types

Table S3.4: ANOVA (Type III tests) for habitat specific indices (ForMI: Forest management intensity index, LUI: Land-use intensity index) and its components. F-values and significance (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$) of models are shown for Shannon Diversity (*H'*), network complexity (H_2) and network generalisation (H_2') (degrees of freedom: *df* = 1 for all factors except region *df* = 2). Arrows indicate significantly increasing (é) or decreasing (ê) effects of land-use components towards the corresponding factors (highlighted in Fig. S3.5). Comprehensive sampling contains n = 150 sites per habitat. Intensive sampling contains four surveys with n = 27 sites per habitat.

(a) Comprehensive survey	Factor[1)]	*H'*	H_2	H_2'
		F-value	*F-value*	*F-value*
Forest	Region	≤ 2.17	≤ 45.35***	≤ 14.34***
	ForMI	1.03	2.43	1.31
	Timber harvest	0.16	1.26	0.33
	Non-native tree species	2.19	5.99*↑	4.34*↓
	Dead wood with saw cuts	2.19	1.17	0.00
Grassland	Region	≤ 2.19	≤ 0.36	≤ 3.74*
	LUI	0.54	2.44	0.96
	sqrt Grazing	2.58	1.35	0.76
	sqrt Fertilization	4.03*↓	8.32**↓	0.02
	Mowing	1.51	1.36	0.52
(b) Intensive survey	Factor[1)]	*H'*	H_2	H_2'
		F-value	*F-value*	*F-value*
Forest	Region	≤ 3.95*	≤ 6.52**	≤ 0.15
	ForMI	0.02	0.39	0.03
	Timber harvest	0.95	0.10	1.24
	Non-native tree species	0.72	0.00	0.01
	Dead wood with saw cuts	0.67	1.83	0.10
Grassland	Region	≤ 6.91**	≤ 1.99	≤ 3.05
	LUI	1.11	2.59	0.24
	sqrt Grazing	2.02	0.89	4.22*↓
	sqrt Fertilization	3.44	4.36*↓	0.10
	Mowing	0.53	0.31	0.46

1) As the factor 'Region', is used to take account for multiple surveys, the highest F-value of all surveys is listed

Table S3.6: Land-use niches for all dung beetle species caught during the intensive survey in forests. The table shows for each species the number of sites where it occurs (Noccur) and the abundance weighted mean with standard deviation (AWM ± SD) for the forest management intensity index and its components. Corresponding P-values highlight the deviance of each species towards the mean land-use level, which results in species-specific fate.

		Forest Management Intensity			Timber Harvest			Non-native Tree Species			Dead Wood With Saw Cuts		
Species	Noccur	AWM ± SD	*P*-value	fate	AWM ± SD	*P*-value	fate	AWM ± SD	*P*-value	fate	AWM ± SD	*P*-value	fate
Anoplotrupes stercorosus	26	1.13 ± 0.76	0.281	neutral	**0.26 ± 0.21**	**0.035**	**loser**	0.36 ± 0.44	0.105	neutral	**0.51 ± 0.31**	**0.041**	**loser**
Aphodius ater	16	**0.92 ± 0.8**	**0.03**	**loser**	**0.25 ± 0.21**	**0.046**	**loser**	0.31 ± 0.45	0.299	neutral	**0.47 ± 0.31**	**0.027**	**loser**
Aphodius coenosus	13	1.27 ± 0.55	0.344	neutral	0.32 ± 0.2	0.442	neutral	0.41 ± 0.46	0.091	neutral	0.52 ± 0.29	0.129	neutral
Aphodius corvinus	6	1.37 ± 1.11	0.276	neutral	0.22 ± 0.19	0.107	neutral	0.35 ± 0.43	0.276	neutral	0.47 ± 0.42	0.104	neutral
Aphodius depressus	26	1.11 ± 0.7	0.219	neutral	0.29 ± 0.24	0.138	neutral	0.33 ± 0.43	0.173	neutral	**0.51 ± 0.31**	**0.023**	**loser**
Aphodius distinctus	7	0.99 ± 0.69	0.197	neutral	0.21 ± 0.22	0.06	neutral	0.32 ± 0.42	0.328	neutral	0.48 ± 0.34	0.116	neutral
Aphodius erraticus	2	1.05 ± 0.17	0.354	neutral	0.34 ± 0.01	0.411	neutral	0.04 ± 0.06	0.415	neutral	0.68 ± 0.11	0.367	neutral
Aphodius fasciatus	7	1.21 ± 0.64	0.476	neutral	**0.16 ± 0.1**	**0.012**	**loser**	0.45 ± 0.5	0.108	neutral	0.47 ± 0.27	0.094	neutral
Aphodius fimetarius	4	**0.65 ± 0.55**	**0.039**	**loser**	0.19 ± 0.27	0.096	neutral	0 ± 0	0.083	neutral	0.47 ± 0.34	0.184	neutral
Aphodius_ granarius	1	0.68 ± NA	0.196	neutral	0.24 ± NA	0.376	neutral	0.05 ± NA	0.381	neutral	0.4 ± NA	0.21	neutral
Aphodius maculatus	6	1.48 ± 0.77	0.14	neutral	0.23 ± 0.16	0.138	neutral	0.5 ± 0.51	0.067	neutral	0.64 ± 0.25	0.389	neutral
Aphodius prodromus	26	1.18 ± 0.71	0.455	neutral	0.29 ± 0.24	0.164	neutral	0.37 ± 0.44	0.07	neutral	**0.51 ± 0.3**	**0.04**	**loser**
Aphodius pusillus	10	1.2 ± 0.85	0.5	neutral	0.22 ± 0.15	0.055	neutral	0.4 ± 0.46	0.13	neutral	0.53 ± 0.29	0.194	neutral
Aphodius rufipes	12	0.93 ± 0.68	0.053	neutral	0.29 ± 0.24	0.26	neutral	0.18 ± 0.32	0.219	neutral	0.52 ± 0.25	0.132	neutral
Aphodius rufus	2	1.83 ± 0.47	0.091	neutral	0.29 ± 0.17	0.492	neutral	0.77 ± 0.29	0.08	neutral	0.83 ± 0.01	0.116	neutral
Aphodius sphacelatus	17	1.12 ± 0.7	0.277	neutral	0.29 ± 0.26	0.192	neutral	0.31 ± 0.44	0.285	neutral	0.53 ± 0.32	0.15	neutral
Aphodius sticticus	21	**0.8 ± 0.75**	**0.002**	**loser**	0.29 ± 0.25	0.187	neutral	0.31 ± 0.42	0.28	neutral	0.52 ± 0.3	0.065	neutral
Aphodius zenkeri	2	0.65 ± 0.63	0.099	neutral	0.22 ± 0.22	0.27	neutral	0 ± 0	0.292	neutral	0.33 ± 0.41	0.091	neutral
Onthophagus coenobita	11	1.41 ± 0.58	0.141	neutral	0.38 ± 0.31	0.254	neutral	0.43 ± 0.47	0.095	neutral	0.61 ± 0.26	0.461	neutral
Onthophagus fracticornis	15	1.27 ± 0.63	0.335	neutral	0.31 ± 0.21	0.355	neutral	0.4 ± 0.43	0.101	neutral	0.51 ± 0.3	0.115	neutral
Onthophagus gibbulus	1	1.56 ± NA	0.301	neutral	0.75 ± NA	0.093	neutral	0.06 ± NA	0.394	neutral	0.75 ± NA	0.393	neutral
Onthophagus joannae	3	1.49 ± 0.36	0.213	neutral	0.35 ± 0.24	0.408	neutral	0.34 ± 0.52	0.274	neutral	0.8 ± 0.04	0.082	neutral
Onthophagus similis	8	1.07 ± 0.65	0.276	neutral	0.3 ± 0.28	0.365	neutral	0.21 ± 0.33	0.38	neutral	**0.42 ± 0.34**	**0.031**	**loser**
Onthophagus vitulus	2	1.01 ± 0.77	0.347	neutral	0.42 ± 0.47	0.269	neutral	0.11 ± 0.07	0.497	neutral	0.49 ± 0.37	0.274	neutral
Trypocopris vernalis	9	1.26 ± 0.64	0.39	neutral	0.22 ± 0.17	0.065	neutral	0.42 ± 0.47	0.122	neutral	0.48 ± 0.31	0.09	neutral
Typhaeus typhoeus	8	1.15 ± 0.67	0.415	neutral	0.24 ± 0.19	0.144	neutral	0.33 ± 0.45	0.323	neutral	0.51 ± 0.32	0.174	neutral

Table S3.5: Land-use niches for all dung beetle species caught during the intensive survey in grasslands. The table shows for each species the number of sites where it occurs (Noccur) and the abundance weighted mean with standard deviation (AWM ± SD) for the land-use intensity index and its components. Corresponding *P*-values highlight the deviance of each species towards the mean land-use level, which results in species-specific fate.

		Land Use Intensity			log Grazing			log Fertilisation			Mowing		
Spec	Noccur	AWM ± SD	*P*-value	fate	AWM ± SD	*P*-value	fate	AWM ± SD	*P*-value	fate	AWM ± SD	*P*-value	fate
Anoplotrupes stercorosus	4	1.32 ± 0.7	0.201	neutral	10.47 ± 4.49	0.272	neutral	3.26 ± 3.9	0.383	neutral	0.5 ± 0.58	0.071	neutral
Aphodius ater	10	1.65 ± 0.91	0.393	neutral	9.77 ± 5.23	0.255	neutral	4.54 ± 5.16	0.09	neutral	1.49 ± 1.44	0.152	neutral
Aphodius coenosus	1	0.92 ± NA	0.1	neutral	10.2 ± NA	0.407	neutral	0 ± NA	0.329	neutral	0 ± NA	0.368	neutral
Aphodius corvinus	1	1.04 ± NA	0.206	neutral	4.58 ± NA	0.312	neutral	0 ± NA	0.368	neutral	1 ± NA	0.356	neutral
Aphodius depressus	8	1.5 ± 0.82	0.334	neutral	7.67 ± 6.29	0.429	neutral	2.57 ± 4.82	0.432	neutral	1.49 ± 1.41	0.19	neutral
Aphodius distinctus	3	1.51 ± 0.41	0.428	neutral	**16.99 ± 3.64**	**0.029**	**winner**	0 ± 0	0.272	neutral	**0.33 ± 0.57**	**0.043**	**loser**
Aphodius erraticus	6	1.61 ± 1.11	0.473	neutral	7.51 ± 2.28	0.393	neutral	**6.55 ± 5.44**	**0.027**	**winner**	**1.98 ± 1.68**	**0.035**	**winner**
Aphodius fasciatus	3	2.12 ± 0.88	0.102	neutral	10.77 ± 3.52	0.262	neutral	6.9 ± 6.19	0.086	neutral	1.66 ± 1.53	0.25	neutral
Aphodius fimetarius	4	1.86 ± 0.53	0.217	neutral	9.99 ± 10.12	0.295	neutral	2.25 ± 4.51	0.414	neutral	1.51 ± 1.29	0.171	neutral
Aphodius fossor	2	1.98 ± 1.49	0.199	neutral	8.3 ± 2.69	0.469	neutral	5.81 ± 8.22	0.125	neutral	2 ± 2.83	0.086	neutral
Aphodius luridus	6	1.53 ± 0.92	0.409	neutral	7.85 ± 2.33	0.466	neutral	3.21 ± 5.13	0.4	neutral	1.17 ± 1.48	0.356	neutral
Aphodius prodromus	25	1.64 ± 0.92	0.4	neutral	8.68 ± 6.42	0.4	neutral	4.4 ± 5.58	0.028	winner	1.27 ± 1.24	0.225	neutral
Aphodius pusillus	6	1.96 ± 1.25	0.108	neutral	7.01 ± 5.06	0.297	neutral	**6.6 ± 7.53**	**0.025**	**winner**	**1.99 ± 1.68**	**0.042**	**winner**
Aphodius rufipes	1	**3.03 ± NA**	**0.028**	**winner**	6.4 ± NA	0.405	neutral	**11.62 ± NA**	**0.029**	**winner**	**4 ± NA**	**0**	**winner**
Aphodius sphacelatus	13	1.91 ± 0.75	0.058	neutral	10.89 ± 5.52	0.086	neutral	**4.88 ± 4.89**	**0.033**	**winner**	1.46 ± 1.39	0.131	neutral
Geotrupes stercorarius	1	1.17 ± NA	0.273	neutral	7.62 ± NA	0.494	neutral	0 ± NA	0.359	neutral	1 ± NA	0.334	neutral
Onthophagus coenobita	13	1.5 ± 0.68	0.304	neutral	9.77 ± 5.8	0.235	neutral	3.1 ± 4.32	0.417	neutral	1.07 ± 0.86	0.413	neutral
Onthophagus fracticornis	11	1.91 ± 0.79	0.076	neutral	8.68 ± 6.83	0.425	neutral	4.51 ± 5.77	0.105	neutral	1.46 ± 1.13	0.099	neutral
Onthophagus gibbulus	1	1.04 ± NA	0.207	neutral	4.58 ± NA	0.326	neutral	0 ± NA	0.363	neutral	1 ± NA	0.334	neutral
Onthophagus joannae	12	1.8 ± 1.17	0.15	neutral	6.86 ± 5.17	0.231	neutral	4.34 ± 5.28	0.091	neutral	1.33 ± 1.16	0.259	neutral
Onthophagus nuchicornis	2	1.13 ± 0.31	0.152	neutral	7.48 ± 10.91	0.469	neutral	0 ± 0	0.41	neutral	0.5 ± 0.71	0.34	neutral
Onthophagus ovatus	7	1.5 ± 0.89	0.333	neutral	8.89 ± 4.08	0.404	neutral	4.31 ± 5.63	0.18	neutral	1.57 ± 1.72	0.131	neutral
Onthophagus similis	7	1.71 ± 0.57	0.349	neutral	11.88 ± 6.93	0.1	neutral	3.52 ± 4.77	0.352	neutral	1.27 ± 0.95	0.438	neutral
Onthophagus verticicornis	6	1.39 ± 1.13	0.201	neutral	6.87 ± 4.97	0.301	neutral	4.45 ± 7.15	0.169	neutral	1.32 ± 1.75	0.357	neutral
Onthophagus vitulus	1	**3.52 ± NA**	**0.011**	**winner**	0 ± NA	0.308	neutral	**15.81 ± NA**	**0.01**	**winner**	**3 ± NA**	**0.013**	**winner**
Trypocopris vernalis	4	1.53 ± 1.08	0.441	neutral	4.17 ± 4.73	0.111	neutral	3.85 ± 7.83	0.33	neutral	1.49 ± 1	0.285	neutral
Typhaeus typhoeus	1	1.37 ± NA	0.439	neutral	10.99 ± NA	0.333	neutral	0 ± NA	0.321	neutral	1 ± NA	0.355	neutral

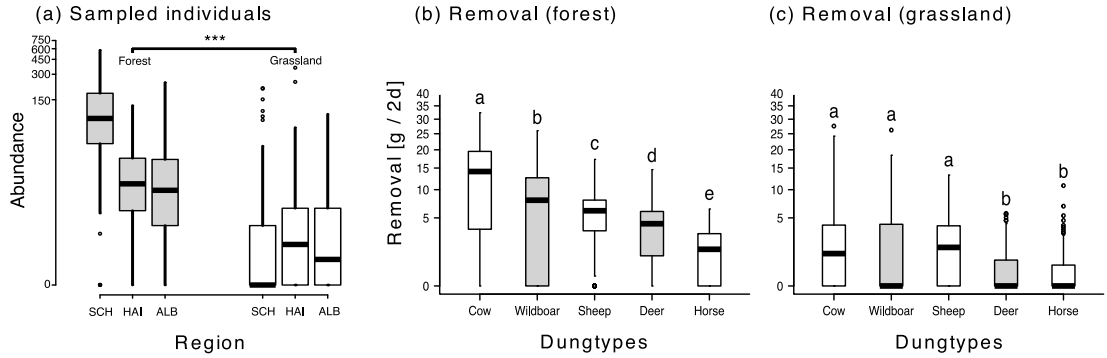


Fig. S3.1. Comprehensive survey (n = 300 sites). Boxplots show the number of beetle individuals caught in each region and habitat (a) and dung removal for all dung types offered in forests (b) and grasslands (c). Note that the y-axis of (a) is log-transformed, while the y-axis of (b) and (c) is square root transformed. Same letters indicate no significant differences (Tukey HSD).

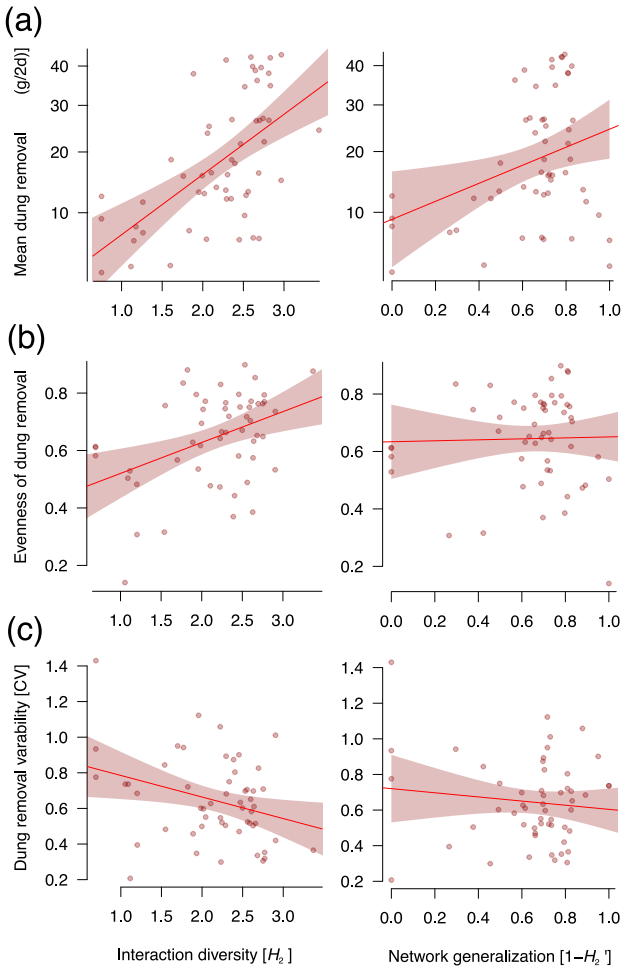
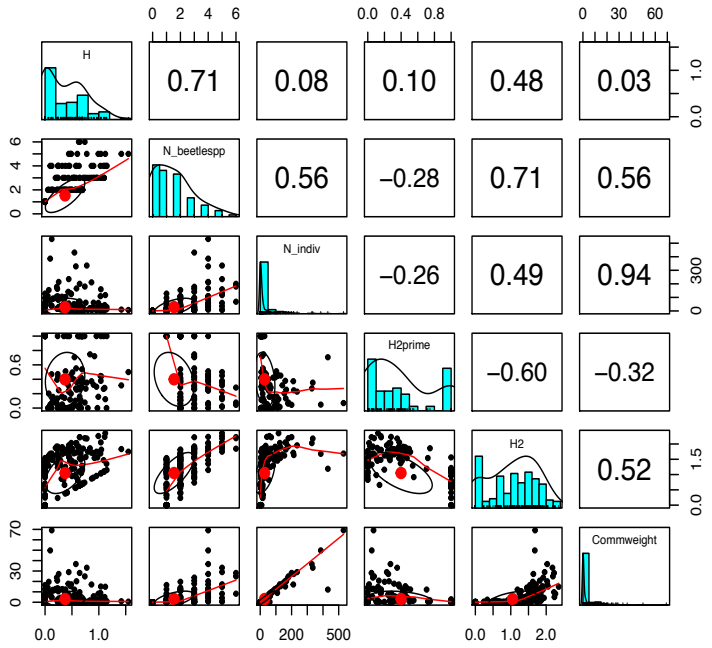


Fig. S3.2. : Intensive Survey. Effects of network properties on the performance and stability of an ecosystem service. (a) The overall rate of dung removal, (b) the evenness of removal rates across six dung types and (c) the temporal stability increased with the complexity (interaction diversity H_2) and generalization (1 – H_2') of dung – beetle networks. Stability is defined as the inverse of the variation (CV) over time(Tilman et al. 2006)

(a)



(b)

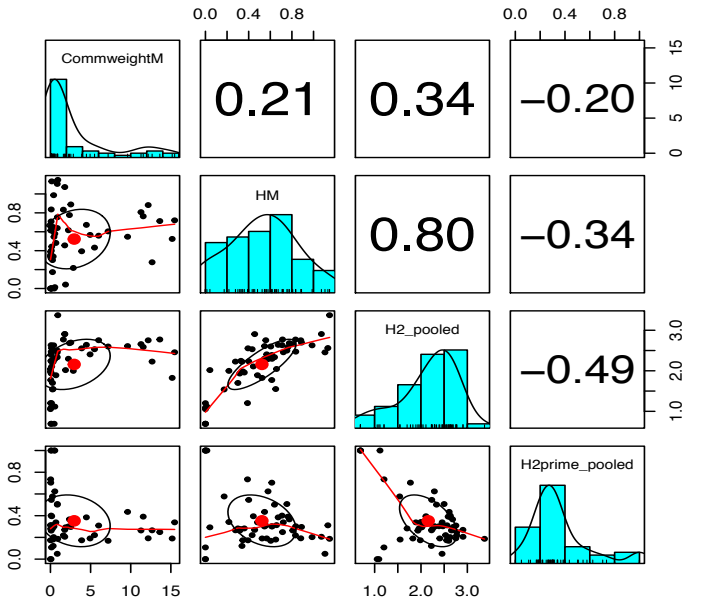


Fig. S3.3. Correlation matrix between the main predictors for (a) comprehensive survey and (b) intensive survey. Across all sites of the comprehensive survey, a higher trophic network complexity H_2 was correlated with beetle abundance (N_indiv), number of beetle species (N_beetlespp) and Shannon diversity H, but also with less resource partitioning of the co-occuring dung beetles in each community reflected by the H_2' that quantifies specialization independent of variation in species' abundance and diversity. Values within boxes represent the Pearsons' r.

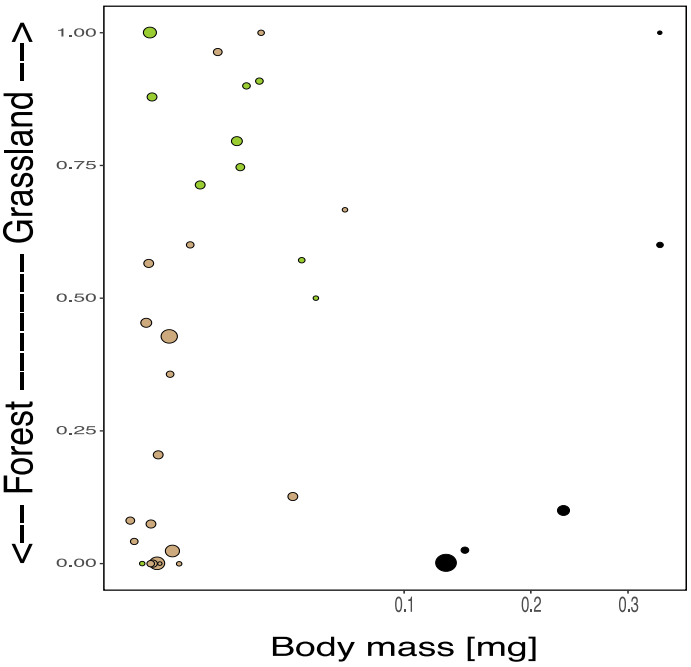


Fig. S3.4. Relationship between body mass and habitat index (proportion of individuals found in grasslands versus forests) across 34 species of dung beetles, each scaled by √total abundance and color-labeled by the three taxa *Aphodius*, Geotrupidae and *Ontophagus*. Grassland preference significantly decreases with body mass in a weighted linear regression (r^2 = 0.19, p = 0.01; species weighted by their putative impact, i.e. the product of √abundance and √biomass), mainly driven by the large and most common species *Anoplotrupes stercorosus* (largest black dot) that almost exclusively occurs in forests.

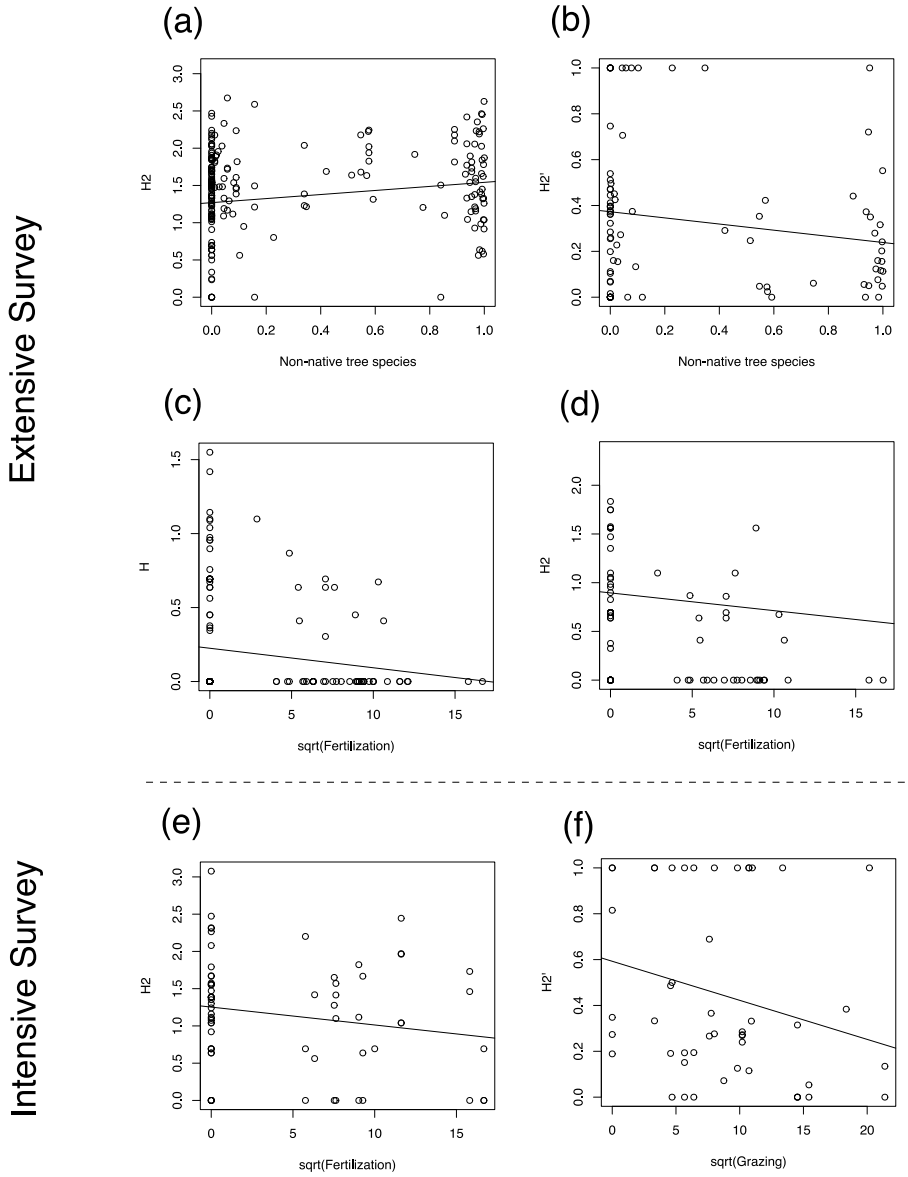


Fig. S3.5. Significant interactions of diversity (*H'*), interaction diversity (H_2) and network generalization (H_2') with management components, highlighted for the extensive survey (a-d) and the intensive survey (e-f).

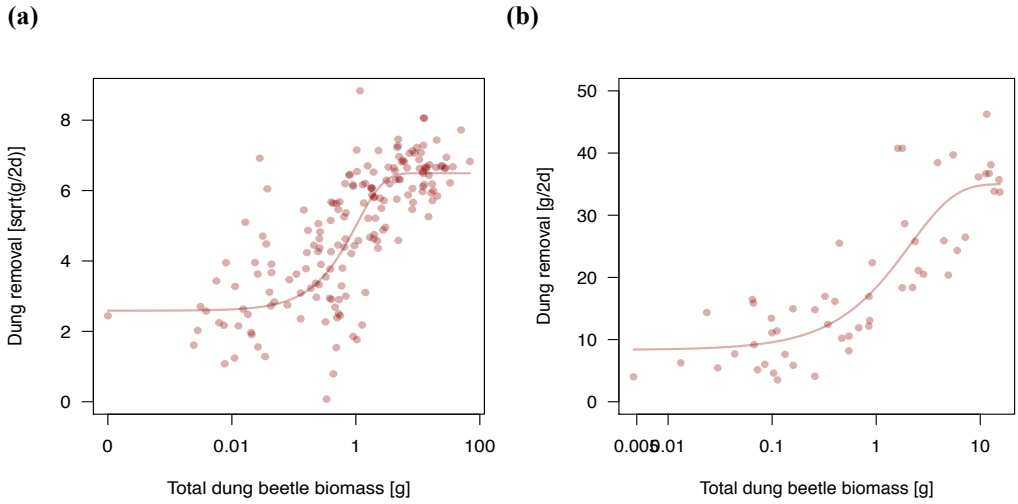


Fig. S3.6. Dung removal rate as a non-linear function of the total biomass (square-root-transformed) of dung beetles independently recorded in each site for (a) the comprehensive survey across all 300 sites and (b) for the mean removal in intensive survey across 54 sites. The following asymptotic regression model was fitted: (a) f(x) = 2.59 + 6.49(1-exp(-x/1.04)) (b) f(x) = 2.94 + 5.91(1-exp(-x/1.68)) where x is the total biomass of dung beetles. All coefficients were significant (mean ± SE: (a) 2.59 ± 0.11, t = 2186, p < 0.001; 6.49 ± 0.19, t = 34.6, p < 0.001; 1.04 ± 0.19, t = 5.4, p < 0.001; (b) 2.94 ± 0.19, t = 15.0, p < 0.001; 5.91 ± 0.24, t = 24.5, p < 0.001; 1.68 ± 0.57, t = 3.0, p < 0.01), and this function explains 64% of the variation in dung removal in (a) and 72 % in (b), indicated by the r^2 of a linear regression between predicted and observed data. The model fitting function was obtained by the command 'drm' (fct = AR.3) in the 'drc' package in R (drc 3.0-1(Ritz et al. 2015)).

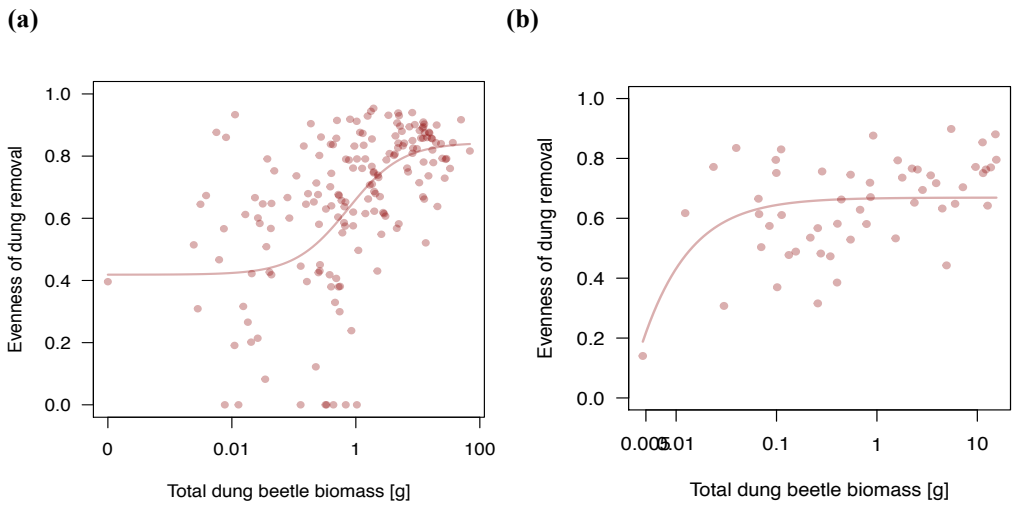


Fig. S3.7. Evenness of removal rates across five types of dung as a non-linear function of the total biomass of dung beetles independently recorded in each site for (a) the comprehensive survey across all 300 sites and (b) for the mean removal in intensive survey across 54 sites. Mean values of evenness and biomass were computed over four surveys per site. The following (shifted) Michaelis-Menten model was fitted: (a) f(x) = 0.42 + (0.84–0.42)/(1+(0.75/x)) (b) f(x) = 0.68/(1+(0.0063/x)) where x is the total biomass of dung beetles. Most coefficients were significant (mean ± SE: (a) 0.41 ± 0.022, t = 18.7, p < 0.001; 0.84 ± 0.040, t = 20.9, p < 0.001; 0.75 ± 0.31, t = 2.5, p = 0.01; (b) 0.68 ± 0.02, t = 29.0, p < 0.001; 0.0063 ± 0.0032, t = 1.9, p = 0.06), and this function explains 30% of the variation in dung removal in (a) and 18 % in (b), indicated by the r^2 of a linear regression between predicted and observed data. The model fitting function was obtained by the command 'drm' (fct = MM.3 and MM.2) in the 'drc' package in R (drc 3.0-1(Ritz et al. 2015)).

Chapter 4

4 Global dung webs: high trophic generalism of dung beetles along the latitudinal diversity gradient

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4.1 Abstract

At the global scale, species diversity is known to strongly increase towards the equator for most taxa. According to theory, a higher resource specificity of species facilitates the coexistence of a larger number of species and has been suggested as an explanation for the latitudinal diversity gradient. However, only few studies support the predicted increase in specialization or even showed contrary results. Surprisingly, analyses for detritivores are still missing. Therefore, we performed an analysis on the degree of trophic specialization of dung beetles. We summarized 41 studies, covering the resource preferences of a total of 990449 individuals, to calculate the dung-specificity in each study region. Our results highlighted a significant (4.1-fold) increase in the diversity of beetles attracted to vertebrate dung towards the equator. However, their resource specificity was low, unrelated to diversity and revealed a highly generalistic use of dung resources that remained similar along the latitudinal gradient.

4.2 Introduction

The latitudinal gradient, particularly the pronounced increase in plant and animal diversity towards the equator, has fascinated biologists for a long time (Darwin 1859, Wallace 1878) and is still a popular research topic (Hillebrand 2004, LaManna et al. 2017, Roslin et al. 2017). A predicted *increase* in specialization towards the tropics has been suggested as an explanation of the high diversity found there (MacArthur 1972). Yet, only a few analyses of trophic or mutualistic interactions have revealed such a trend (Olesen and Jordano 2002, Dyer et al. 2007). On the contrary, specialization of pollinators and frugivores (Schleuning et al.

2012), and bark beetles (Beaver 1979) on their host plants decreases towards the equator, and the high specialization level of herbivores and host-parasitoid networks remains similar along the latitudinal gradient (Novotny et al. 2006, Morris et al. 2014, Forister et al. 2015).

At the base of every food web, decomposers process organic material and provide a nutritional basis for higher trophic levels. Whereas plant litter is abundant, but comparably poor in nutrient quality for consumers, animal carcasses or dung – although representing already processed food – represent high quality resources, with high levels of all essential nutrients such as amino acids, fatty acids, and sterols (Enser et al. 1996, Carter et al. 2007, Frank et al. 2017a). Consequently, competition among detritivores for carcasses and dung can be pronounced, an important prerequisite for niche differentiation among competing species. Yet, there are no assessments of global specialization patterns for detritivores on their resources.

Dung beetles (Scarabaeoidea) are known to use a wide range of dung types among a few other resources such as carcasses, humus, and fungi. However, most species feed on vertebrate dung as their main resource (Hanski and Cambefort 1991). These beetles are almost ubiquitous in all climatic zones, including hot spots with over 80 sympatric species in tropical forests and savannahs (Hanski and Cambefort 1991, Davis 2000, Feer and Hingrat 2005, Barragan et al. 2011). Hence, we focused on this cosmopolitan superfamily of insects, which evolved a detritivorous life-style over a hundred million years ago (Krell 2006, Philips 2011), to conduct a meta-analyses of their resource-specificity. We compiled datasets from across the globe on the distribution of dung beetles occurring on two or more vertebrate dung types. We assessed trends in species diversity and dung type specialization along the latitudinal gradient and with increasing altitude. Although highly specialized tropical dung beetle species exist, often utilizing resources other than vertebrate feces and differentiated in several other niche dimensions, the global analysis for beetle communities captured with commonly available mammalian dung revealed a highly generalistic use of dung resources. This study highlights contrary findings to classical niche theory and fills a gap of current knowledge for detritivores as a basic trophic level.

4.3 **Material and Methods**

We assembled datasets from the literature that included dung beetles trapped by or surveyed from two or more types of dung in the same study area. Many surveys of dung

beetle diversity include only a single type of dung and so could not be used in this analysis. For the comparability of the dung resource specificity, we excluded carrion and vegetable matter as resources in six studies. We required studies that provided the total abundance of each dung beetle species on each dung type, excluding studies where species were pooled at the genus level, or where abundances were only summarized across dung types. For studies that sampled within different habitats (i.e. forests and grasslands), but with the same dung types, we conducted separate analyses to account for habitat-specific dung beetle communities. In total, we found 29 papers published between 1966 and 2017. In addition, we also included 11 of our own unpublished datasets. All datasets including geographic coordinates, the total number beetle species, and the degree of specialization (see below) are summarized in Table S4.1 (Supporting Information); additional information for separate subsets of the data (networks) is also included therein.

Data analysis

For each study we calculated the rarefied effective Shannon diversity of beetles ($e^{H'}$) (based on 100 permutations), as the studies showed variation in sampling effort, beetle density, and consequently the total number of individuals collected. We set a minimum value of 100 individuals for rarefaction. In six networks (from three studies, see Table S4.1 in Supporting Information) the number of individuals was below this threshold and so the non-rarefied effective Shannon diversity was used for these networks.

As a measure of complementary specialization in networks, the standardized two-dimensional Shannon entropy H_2' (Blüthgen et al. 2006) was calculated to quantify the degree of resource partitioning across dung beetle species: the minimum $H_2' = 0$ is defined for the case where all dung beetle species are utilizing different dung types in similar proportions, whereas the maximum level $H_2' = 1$ is reached if all dung types are used as exclusively as possible by different beetle species. Minimum and maximum H_2' were defined by heuristically re-distributing the beetle individuals across dung types, but fixing the marginal totals of the beetle species × dung type contingency table (with number of individual beetles as cell entries); hence, the total abundances per beetle species and per dung type are maintained. In a null model based on Patefield's algorithm, also based on fixed marginal totals, the individuals were randomly distributed 10^5 times, allowing us to assess whether H_2' was significantly higher than random (Blüthgen et al. 2006). To compare the degree of specialization on each type of dung *i*, we also computed the species-level Kullback-Leibler

distance d_i' that is related to H_2' (Blüthgen et al. 2006). For each dung type i, d_i' describes the exclusiveness of the beetle species attracted, i.e. d_i' = 0 if the dung type i is used by the same set of beetle species as all other dung types offered and in similar proportions, while d_i' = 1 if dung type i attracts only exclusive beetle species.

We also quantified the relative attractiveness of dung types, standardized for each network as N_i/N_{max}, where N_i is the total number of beetle individuals recorded on dung type i and N_{max} the maximum number of beetles found for any dung type in this network. In the same way, we quantified the relative number of species attracted as S_i/S_{max}, with S_i being the beetle species richness for dung type i and S_{max} the maximum richness for any dung type in this network. Note that there is no "standard" dung or methodology across all studies, which limits the comparability, as each dung type is evaluated in a variable context of other dung types offered in the same study; nevertheless, this analysis should be useful for understanding the role of different dung types across the datasets. We thus tested whether these parameters change along the latitudinal gradient for five of the most attractive dung types i.e. with the largest number of sampled beetles: human, wildebeest, donkey, sheep and pig.

We used a linear mixed effects model to test for effects on a latitudinal gradient, accounting for potential linear and quadratic effects of altitude. We used thus employed altitude, altitude^2 and absolute latitude as fixed factor. To account for non-independence of data within studies providing multiple networks from the same region, we used the region(s) of each study (geographic coordinates) as random factor. We used this model structure to test the following response variables: resource specificity (H_2'), rarefied effective Shannon diversity ($e^{H'}$), specialization levels for dung types (d_i'), individual and species based attractiveness of dung (N_i/N_{max} ; S_i/S_{max}).

Data analyses were conducted with the statistical software R 3.3.2 (R Core Team 2016). For community analyses (species richness, Shannon diversity) we used the R package 'vegan' (Oksanen et al. 2007).

4.4 **Results**

Dung beetle networks (110 networks from 41 studies) in this analysis covered 6 continents and 26 countries, ranging from 60° N to 34° S and 151° E to 103° W (Fig. 4.1, Table 4.1) with a high density of studies in Central Europe (Fig. 4.1 b).

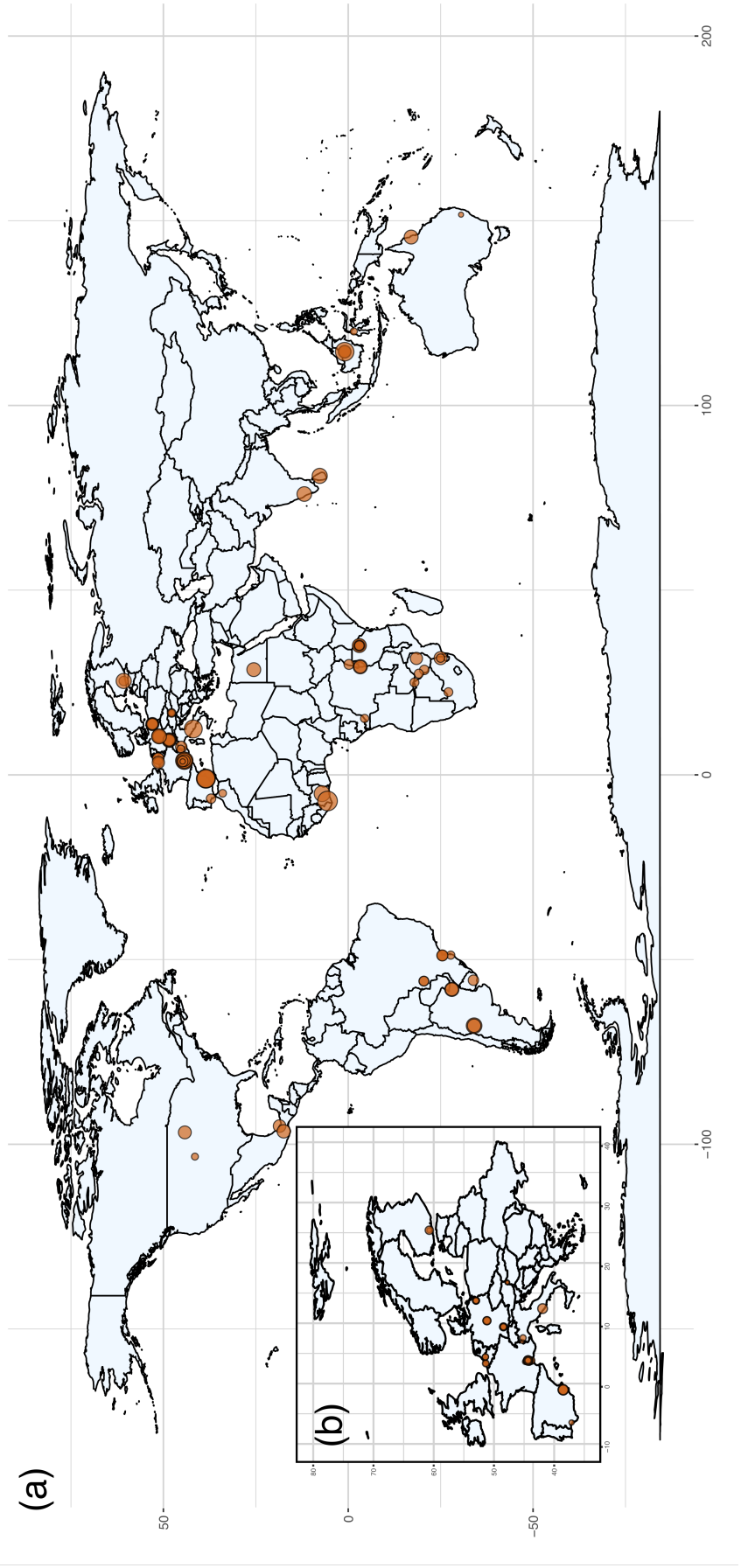

Figure 4.1: Global (a) and European (b) map for beetle – dung networks represented in this study. Dot positions represent the sampling region, their sizes increase with the degree of dung specificity H_2'.

Table 4.1: Number of networks (N_{webs}), total number of individulas ($N_{individuals}$), mean number of beetle species (S), mean number of dung types (N_{dung}) and mean resource specificity (H_2') per network for each reference used in the present study.

Reference	N_{webs}	$N_{individuals}$	S	N_{dung}	H_2'
(Cambefort 1991a)	1	52220	123	2	0.48
(Barbero et al. 1999)	1	2244	10	4	0.19
(Bogoni and Hernandez 2014)	1	426	17	4	0.06
(Carpaneto et al. 2005)	1	1708	20	2	0.65
(Carpaneto et al. 2010)	1	2024	25	3	0.12
(Correa et al. 2016)	2	7089	43	3	0.11
(Davis 1994)	1	14648	52	3	0.33
(Davis et al. 2010)	1	63934	47	4	0.08
(Donovan 1979)	1	456357	6	5	0.01
(Dormont et al. 2004)	3	5672	21.67	2	0.19
(Dormont et al. 2007)	4	6353	14.25	4	0.13
E. Slade & E. Raine, unpubl. data	3	1141	24	8	0.15
(Errouissi et al. 2004)	3	6684	9.67	2	0.04
(Estrada et al. 1993)	1	1567	22	2	0.25
(Foster 1993)	8	61829	18.5	2.13	0.13
(Frank et al. 2017b) and unpubl. data	22	19348	34	7.36	0.23
(Galante and Cartagena 1999)	4	955	11	2	0.63
(Gardiner 1995)	3	61112	35	3	0.14
(Hewavithana et al. 2016)	1	454	22	5	0.41
(Jay-Robert et al. 2008)	6	7485	20.67	2	0.37
(Kessler et al. 1974)	1	2429	14	2	0.26
E. Slade, L. Yuen Chiew, C. S. Vairappan, unpubl. data	6	9123	39.34	9.34	0.2
(Cambefort and Walter 1991) and unpubl. data	1	1614	73	5	0.71
(Martín-Piera and Lobo 1996)	1	2477	35	9	0.1
(Martinez and Suarez 2006)	1	2574	6	2	0.3
(Milotic et al. 2017)	4	7845	17	3	0.18
(Morelli et al. 2002)	1	1846	12	2	0.14
(Nibaruta 1982)	4	10123	17.5	4.5	0.27
(Paetel 2002)	2	36032	76	5.5	0.18
(Rainio 1966)	2	16190	22.5	3	0.26
(Ricou 1981)	2	8837	13	2	0.08
(Wurmitzer et al. 2017) (Austria) and unpubl. data	6	14604	7.34	3	0.03
(Shahabuddin et al. 2010)	1	1429	28	2	0.04
(Cambefort and Walter 1991) and unpubl. data	1	3108	72	3	0.82
(Tshikae et al. 2008)	1	68393	67	4	0.08
(Vernes et al. 2005)	1	541	11	5	0.33
(Vinod and Sabu 2007)	1	2657	46	2	0.38
P. Walter 1978 and unpubl. data	1	18932	100	5	0.06
(Whipple and Hoback 2012b)	1	7395	15	11	0.03
(Wurmitzer et al. 2017) (Argentina) and unpubl. data	4	1050	13.75	9	0.34

As expected, the diversity of dung beetles (rarefied effective Shannon diversity $e^{H'}$) significantly increased towards the tropics (Table 4.2), with a 4.1-fold increase from the highest latitude (60.7°) to near the equator (0.2°) (Fig. 4.2a). Altitude did not have a significant effect on beetle diversity across the studies used for this analysis (although some studies reported a decrease in dung beetles trapped with a single dung type with an increase in altitude within a region, e.g. Escobar et al. (2005) and Lobo et al. (2007) (Table 4.2). Dung beetle – resource specificity (H_2') was relatively low (0.22 ± 0.17, range). This high level of generalization remained constant with latitude as well as with altitude (Table 4.2). Moreover, H_2' was unrelated to (rarefied) Shannon diversity ($F_{1,41} = 0.16$, $p = 0.696$ (Fig. 4.2b).

Table 4.2: Rarefied effective Shannon diversity ($e^{H'}$) increased significantly with declining latitude, while altitude had no effect. The beetles' resource specificity (H_2') showed no significant change for both, altitude and latitude.

	rarefied $e^{H'}$		H_2'	
	$F_{1,45}$	p	$F_{1,45}$	p
Altitude	0.78	0.38	1.36	0.25
Altitude^2	0.73	0.39	0.03	0.86
|Latitude|	30.9	0.0001	2.17	0.15

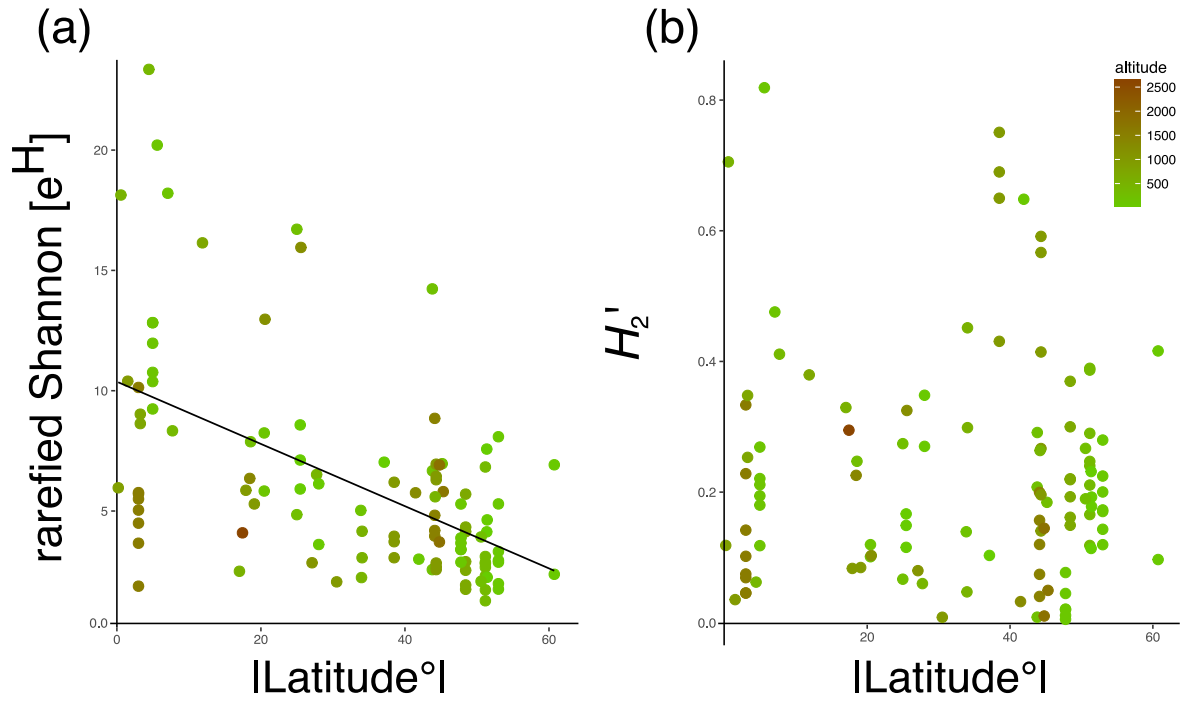

Figure 4.2:Changes in (a) rarefied effective Shannon diversity ($e^{H'}$ for 100 individuals per network) and (b) dung-type specificity H_2' along an absolute latitudinal gradient (0° = equatorial level, northern and southern hemisphere is plotted up to 60°). Colouring of dots represents the altitude of the study region.

Across the different studies, human dung attracted the highest number of beetle individuals, followed by feces from several herbivorous or omnivorous large mammals. Among carnivorous mammals, lion and lynx feces had an intermediate attractiveness, whereas dung from puma, wolf and bear were much less attractive (Fig. 4.3a). Most of these more attractive dung types also attracted the largest number of species (Fig. 4.3b). Variation in standardized attractiveness and species richness was highly significant across dung types (N_i/N_{max}: $F_{19, 286}$=4.3, $p < 0.0001$; S_i/S_{max}: $F_{19, 286}$=15.0, $p < 0.0001$; focusing on 20 dung types that were each represented in at least five networks). Yet, most of the focal dung types attract a largely representative spectrum of beetle species in similar proportions, resulting in relatively low specialization levels (d_i') (Fig. 4.3c) that were similar across dung types ($F_{19,286}$ =1.1, p = 0.31). Additionally, all parameters (attractiveness, richness or d_i') remained similar along the latitudinal and altitudinal gradients for each of the four dung types used in at least 10 regions (all $p \geq 0.30$), except for an increase of N_i/N_{max} for sheep dung with latitude ($F_{1,12}$ = 11.6, p = 0.005) (Figure S4.1, Supporting Information).

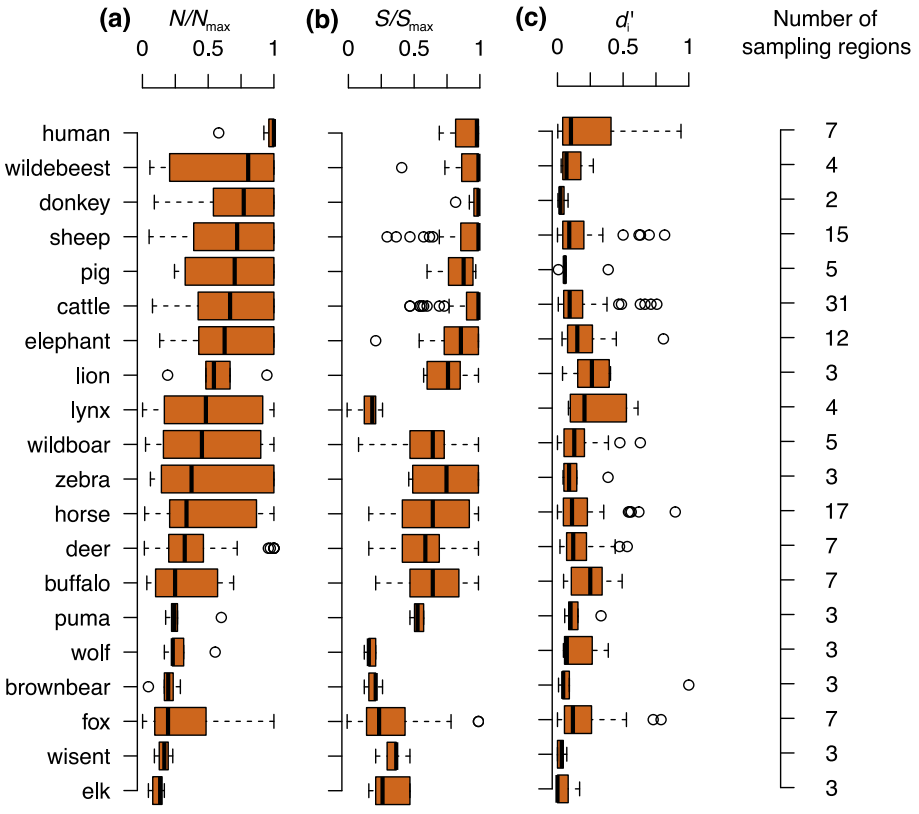

Figure 4.3: Dung beetle attraction to the 20 most commonly used dung types (represented in at least 5 networks). (a) The popularity of dung was expressed as the number of beetle individuals N_i per dung type relative to the most attractive dung type in each study (N_i/N_{max}), and (b) the number of beetle species S_i was expressed in relation to the maximum found per study accordingly (S_i /S_{max}). (c) The degree of specialisation of each dung type (d_i') quantifies the relative composition of dung beetle species in comparison to the other dung types in the study.

4.5 **Discussion**

The latitudinal gradient for species diversity and interaction-specificity is the subject of ongoing research and comparative approaches (Beaver 1979, Dyer et al. 2007, Schleuning et al. 2012, LaManna et al. 2017). Here, we analysed, for the first time and on a global scale, the resource specificity of dung beetles. These important detritivores, are present in all climatic zones, are key biological indicators in monitoring programs (Scholtz et al. 2009), and are of vital importance for many ecosystem functions and services (Nichols et al. 2008, Beynon et al. 2015).

We found dung beetle interaction networks differed widely in their degree of specialization ($0.01 \leq H_2' \leq 0.76$), but found no evidence for an increase in specialization towards the equator (Figs. 4.1 & 4.2). Dung beetles may be expected to be generalists in higher latitudes due to the low diversity of dung available to specialize on. Thus, for a species of this particular, detritivorous group, becoming a specialist can incur trade-offs, and the low specialization in the tropics may highlight the beetles' ability to opportunistically respond to available resources. Studies on dung beetles differ strongly in their sampling approach and particularly in the dung types used. Our analysis was confined to studies that used at least two different dung types, which is a relatively small subset of dung beetle studies. The dung types analysed included livestock and wild animals, and ranged from commonly used cow and human dung to region-specific fauna. Cow and human dung are often used as standardized dung types as they are thought to attract a large part of the dung beetle fauna (Hanski and Cambefort 1991, Whipple and Hoback 2012b, Marsh et al. 2013), which was confirmed in our analysis (Fig. 4.3). Including dung from non-native animals and/or livestock might bias the analysis towards relatively opportunistic, generalist species. However, the diversity of dung types used for this analysis had no effect on the degree of specialization, and native dung did not attract a more specific beetle fauna than other dung types (Fig. 4.3).

This study corroborates the commonly held assumption that most dung beetles are opportunistic and generalized, using a broad range of vertebrate dung types (Hanski and Cambefort 1991). We did not look at specializations of dung beetles outside of vertebrate dung, and we acknowledge that there are dung beetle species that are highly specialized on particular types of dung, such as sloth faeces (Young 1981), or other food items, such as millipede carcasses (Schmitt et al. 2004), or, rarely, vertebrate carrion (Larsen et al. 2006, Scholtz et al. 2009).

Dung beetle resource-specificity did not change with altitude. All studies included in the analysis were conducted below 2000 m a.s.l. (except Martinez & Suarez 2006 at ~2600 m a.s.l.). The occurrence of dung beetles is driven firstly by the spatial distribution of dung producing mammals, and secondly by the climatic conditions which constraint all ectothermic insects (Kuhn 2010, Bogoni et al. 2016). Thus, the major drivers of altitudinal limitations in dung beetle occurrence are the thermal conditions and altitudinal shifts in vegetation affecting the diversity of mammals the dung beetles rely on.

Theory suggests that specialization on resources provides niche partitioning and thus potentially enhances coexistence and species diversity (McKane et al. 2002). However, we found dung beetle communities with high diversity but a low degree of resource specificity (Tshikae et al. 2008, 67 species, H_2' = 0.12), and communities with low diversity but with high specificity (Hewavithana et al. 2016, 22 species, H_2' = 0.41). Across the gradient in dung beetle diversity, the degree of specialization was similar (Fig. 4.2b). This suggests that niche dimensions other than resource selectivity may be important to facilitate the coexistence of dung beetle species. These might be temporal patterns, such as variation in activity period (Feer and Pincebourde 2005), or differences in dung discovery (Jacobs et al. 2008), or spatial differentiation in different (micro-)habitats (Hanski and Cambefort 1991, Mehrabi et al. 2014). Such differentiation in temporal activity and/or environmental conditions might increase towards the tropics, and in more diverse communities, but remains to be tested. Given that dung beetle species within a community show pronounced overlap in utilization of dung resources, higher beetle diversity may improve functional complementarity and redundancy within a community (Finke and Snyder 2008), resulting in increases in the rates, stability, and resilience of ecosystem functions and services provided by dung beetles.

Conclusion

We quantified the beetles' dung-resource specificity (i.e. the degree of specialization in trophic networks characterized by the distribution of beetle species among dung types) across all available studies using two or more dung types. The analysis confirms a highly generalistic use of dung by dung beetle communities, at a comparable level as reported for generalized frugivores or nectar-seeking ants (Blüthgen et al. 2007, Dyer et al. 2007), regardless of latitude or altitude. Additionally, we found no correlation between dung beetle specificity and dung beetle diversity. Although competition across beetle species for dung resources can be severe, and different life-history strategies exist to rapidly utilize and

monopolize portions of dung piles, increased specialization does not provide an explanation why so many dung beetle species coexist in a given habitat. This unprecedented analysis is a first step to acquire global patterns for the most basal, trophic level, possibly stimulating global comparisons of other detritivorous systems.

Acknowledgements

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4.6 **Supporting Information to Chapter 4**

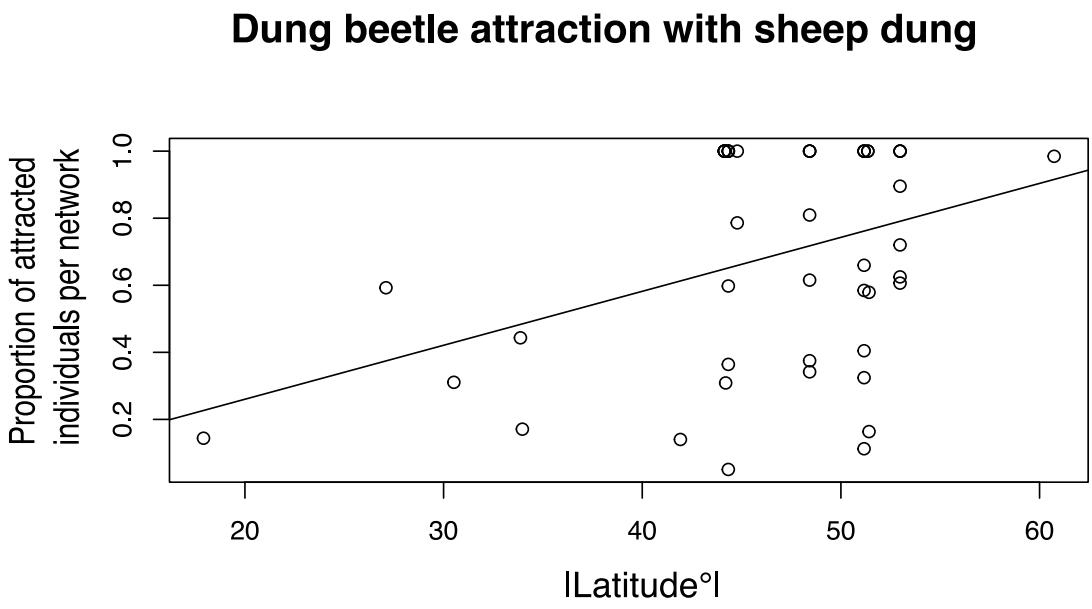


Figure S4.1: Dung beetle attraction showed an increase of the proportion of attracted individuals per network with increasing latitude for sheep dung.

Table S4.1 (1/2): All datasets including geographic coordinates, the total number beetle species, and the degree of specialization.

Reference / year of data sampling	N beetles	N beetle species	N dung types	beetle-resource specificity (H2')	rarefied species richness	SD for rarefied species richness	Shannon diversity	rarefied Shannon diversity	SD for rarefied Shannon diversity	dung types	herbivore	omnivore	carnivore	sampling area	latitude	longitude	~ altitude (m a.s.l.)
Davis 1994	14648	52	3	0.33	27.1	2.54	20.2	15.95	2	cattle, horse, pig	x	x	x	Pretoria, South Africa	25°33'6" S	28°29'0" E	1250
Davis et al. 2010	63934	47	4	0.08	9.89	1.84	3.23	2.85	0.43	pig, elephant, cattle, sheep	x	x	-	Tswalu Kalahari Reserve, South Africa	27° 6'0"S	22°23'6"E	1100
Carpaneto et al. 2010	2024	25	3	0.12	13.32	1.39	6.46	5.97	0.7	buffalo, hippo, warthog	x	x	-	Queen Elizabeth National Park, Uganda	0°11'2" S	0°11'2" E	900
Cambefort & Walter 1991	1614	73	5	0.71	29.97	3.06	23.37	18.13	2.43	human, elephant, duiker, civet, river hog	x	x	-	Makokou, Gabun	0°33'44"N	12°50'02.8"E	530
Shahabuddin et al. 2010	1429	28	2	0.04	17.28	1.71	11.9	10.4	0.98	cattle, anoa	x	-	-	Lore Lindu National Park, Sulawesi	1°30'24" S	120°02'11" E	1000
Vinod & Sabu 2007	2657	46	2	0.38	24	2.06	19.65	16.15	1.47	elephant, gaur	x	-	-	Pothumoola, India	11°53'00.0"N	76°01'00.0"E	750
Vernes et a. 2005	541	11	5	0.33	7.03	1.36	2.64	2.5	0.31	Isoodon macrourus, Bettongia tropica, Uromys caudimaculatus, Perameles nasuta, Dasyurus hallucatus	x	x	-	Davies Creek, Australia	17° 1' S	145°35' E	550
Martínez & Suarez 2006	2574	6	2	0.3	5.74	0.44	4.21	4.09	0.33	cattle, horse	x	-	-	Sierra de Juarez, Mexico	17°26'31.1" N	96°30'13.2" W	2600
Tshikae et al. 2008	68393	67	4	0.08	12.29	2.02	6.58	5.86	0.8	cattle, sheep, pig, elephant	x	-	x	Chobe National park, Botswana	17°54'52.4" S	25°0'19.4" E	1000
Gardiner 1995, PhD Thesis	5306	15	3	0.23	11.07	1.08	6.77	6.35	0.58	cattle, buffalo, elephant	x	-	-	Wedza, Simbabwe	18°27'00.0"S	31°31'00.0"E	1400
Estrada et al. 1993	1567	22	2	0.25	13.36	1.43	8.69	7.88	0.79	coati, howler monkey	x	x	-	Veracruz, Mexico	18°34'00.0"N	95°04'00.0"W	300
Gardiner 1995, PhD Thesis	28092	41	3	0.09	16.61	2.11	6.24	5.29	0.86	cattle, wildebeest, elephant	x	-	-	Ngamo Forest Area, Simbabwe	19°05'00.0"S	27°24'00.0"E	1000
Correa et al. 2016	4345	43	3	0.12	15.44	1.93	9.33	8.25	1.02	carrion, cow, human, pig	x	x	-	Aquidauana, Brazil, savanna	20°27'28"S	55°50'16"W	150
Correa et al. 2016	2744	43	3	0.1	12.61	1.83	6.4	5.83	0.72	carrion, cow, human, pig	x	x	-	Aquidauana, Brazil, exotic pasture	20°27'28"S	55°50'16"W	150
Gardiner 1995, PhD Thesis	27714	49	3	0.1	23.96	2.51	16.13	12.97	1.61	cattle, wildebeest, white rhino	x	-	-	Matobo National Park, Simbabwe	20°34'00.0"S	28°25'00.0"E	1200
Paetel 2002, PhD Thesis	19974	85	6	0.28	26.14	2.59	21.47	16.71	1.76	elephant, rhino, zebra, buffalo, wildebeest, impala	x	-	-	Kruger park, South Africa	24°59'00.0"S	31°36'00.0"E	280
Paetel 2002, PhD Thesis	16058	67	5	0.07	14.68	2.27	5.7	4.85	0.71	elephant, zebra, buffalo, giraffe, impala	x	-	-	Kruger park, South Africa	24°59'00.0"S	31°36'00.0"E	280
Eleanor Slade & Elizabeth Raine 2015/16, unpublished data	315	24	8	0.12	11.67	1.07	7.57	7.12	0.54	human, giant ant eater, black capuchin, fox, jaguar, ocelot, grison, raccoon	x	x	x	Serra do Mar, Paraná state, Brazil	25°27'11.0"S	48°52'57.0"W	30
Eleanor Slade & Elizabeth Raine 2015/16, unpublished data	511	24	8	0.15	13.61	1.21	9.22	8.58	0.7	human, giant ant eater, black capuchin, fox, jaguar, ocelot, grison, raccoon	x	x	x	Serra do Mar, Paraná state, Brazil	25°27'11.0"S	48°52'57.0"W	30
Eleanor Slade & Elizabeth Raine 2015/16, unpublished data	315	24	8	0.17	13.19	1.47	6.41	5.92	0.68	human, giant ant eater, black capuchin, fox, jaguar, ocelot, grison, raccoon	x	x	x	Serra do Mar, Paraná state, Brazil	25°27'11.0"S	48°52'57.0"W	30
Bogoni et al. 2014	426	17	4	0.06	12.51	1.52	6.97	6.51	0.64	puma, fox, capuchin, tapir	x	x	x	Santo Amaro da Imperatriz, Santa Catarina, Brazil	27°44'S	48°48'W	480
Wurmitzer et al. 2017 (Argentina) and unpublished data	402	18	9	0.35	11.6	1.39	3.85	3.61	0.47	buffalo, carpincho, mara, guanaco, horse, lion, puma, fox, monkey	x	x	x	Corrientes, Argentina	28°01'40"S	58°04'02"W	75
Wurmitzer et al. 2017 (Argentina) and unpublished data	249	18	9	0.27	12.46	1.21	6.39	6.13	0.54	buffalo, carpincho, mara, guanaco, horse, lion, puma, fox, monkey	x	x	x	Corrientes, Argentina	28°01'40"S	58°04'02"W	75
Foster 1993, PhD Thesis	14355	46	2	0.23	17.41	2.7	6.01	5.04	1.02	wildebeest, elephant	x	-	-	Serengeti National Park, Tansania	3°00'00.0"S	35°00'00.0"E	1600
Foster 1993, PhD Thesis	33672	46	2	0.05	22.93	2.51	12.86	10.14	1.52	wildebeest, zebra	x	-	-	Serengeti National Park, Tansania	3°00'00.0"S	35°00'00.0"E	1600
Foster 1993, PhD Thesis	106	6	3	0.33	6	0	5.71	5.71	0.04	ruminant, zebra, elephant	x	-	-	Serengeti National Park, Tansania	3°00'00.0"S	35°00'00.0"E	1600
Foster 1993, PhD Thesis	7294	19	2	0.07	11.01	1.36	5.89	5.5	0.54	wildebeest, ostrich	x	-	-	Serengeti National Park, Tansania	3°00'00.0"S	35°00'00.0"E	1600
Foster 1993, PhD Thesis	4179	13	2	0.05	9.59	1.11	3.95	3.66	0.47	wildebeest, Thomson´s gazelle	x	-	-	Serengeti National Park, Tansania	3°00'00.0"S	35°00'00.0"E	1600
Foster 1993, PhD Thesis	249	3	2	0.08	3	0	1.88	1.88	0.13	wildebeest, cattle	x	-	-	Serengeti National Park, Tansania	3°00'00.0"S	35°00'00.0"E	1600
Foster 1993, PhD Thesis	835	8	2	0.14	7.94	0.24	5.88	5.77	0.42	cattle, zebra	x	-	-	Serengeti National Park, Tansania	3°00'00.0"S	35°00'00.0"E	1600
Foster 1993, PhD Thesis	1139	7	2	0.1	6.44	0.66	4.64	4.5	0.33	cattle, elephant	x	-	-	Serengeti National Park, Tansania	3°00'00.0"S	35°00'00.0"E	1600
Nibaruta 1982, PhD Thesis	3076	25	3	0.35	13.89	1.09	9.69	9.02	0.77	bos taurus autochthon, bos indicus, bos taurus europa	x	-	-	Burundi	3°15'00.0"S	29°16'00.0"E	770
Nibaruta 1982, PhD Thesis	6250	25	3	0.25	14.3	1.28	9.29	8.63	0.78	bos taurus autochthon, bos indicus, bos taurus europa	x	-	-	Burundi	3°15'00.0"S	29°16'00.0"E	770
Donovan 1979, PhD Thesis	456357	6	5	0.01	2.58	0.64	2.07	2.06	0.07	cattle, horse, human, kangaroo, sheep	x	x	-	Armidale, Australia	30°31'00.0"S	151°39'00.0"E	1000
Morelli et al. 2002	1846	12	2	0.14	9.81	0.92	5.32	5.03	0.52	cattle, sheep	x	-	-	Cerro Colorado, Uruguay	33°52'6.25" S	55°35'9.50" W	200
Errousi et al. 2004	1439	7	2	0.05	5.92	0.72	2.33	2.23	0.27	cattle, sheep	x	-	-	Fes, Morocco	33°58'0" N	4°59'0" W	500
Wurmitzer et al. 2017 (Argentina) and unpublished data	97	6	9	0.45	6	NA	3.06	3.06	NA	buffalo, carpincho, mara, guanaco, horse, lion, puma, fox, monkey	x	x	x	Nacunan, Argentina	34°02'42"S	67°54'32"W	560
Wurmitzer et al. 2017 (Argentina) and unpublished data	302	13	9	0.3	7.8	1.21	4.25	4.16	0.37	buffalo, carpincho, mara, guanaco, horse, lion, puma, fox, monkey	x	x	x	Nacunan, Argentina	34°02'42"S	67°54'32"W	560
Martín-Piera & Lobo 1996	2477	35	9	0.1	16.05	1.78	7.85	7.03	0.98	horse, cattle, wild boar, bager, deer, fallow deer, lynx, fox, human	x	x	x	Coto de Doñana, National Park, Spain	37°07'00.0"N	6°27'00.0"W	2
Galante et al. 1999	319	14	2	0.43	8.66	1.67	3.9	3.72	0.39	cattle, rabbit	x	-	-	Sierra de Salinas/Sieera de Onil, Alicante, Spain	38°29'56.43" N	1° 1'53.26" W	1000
Galante et al. 1999	43	8	2	0.69	8	NA	3.95	3.95	NA	cattle, rabbit	x	-	-	Sierra de Salinas/Sieera de Onil, Alicante, Spain	38°29'56.43" N	1° 1'53.26" W	1000
Galante et al. 1999	260	9	2	0.75	7.29	0.97	3.17	3.06	0.27	cattle, rabbit	x	-	-	Sierra de Salinas/Sieera de Onil, Alicante, Spain	38°29'56.43" N	1° 1'53.26" W	1000
Galante et al. 1999	333	13	2	0.65	10.85	0.9	6.47	6.2	0.51	cattle, rabbit	x	-	-	Sierra de Salinas/Sieera de Onil, Alicante, Spain	38°29'56.43" N	1° 1'53.26" W	1000
Walter 1978, postdoctoral Thesis and unpublished data	18932	100	5	0.06	33.42	2.86	31.87	23.35	2.49	human, elephant, lion, hyena, marshbuck	x	x	x	Kinshasa, Congo	5°06'31.0"S	15°15'58.7"E	430
Eleanor Slade & Li Yuen 2016/17, unpublished data	791	37	10	0.2	20.16	1.94	14.7	12.82	1.12	cattle, sun bear, elephant, bear cat, rhino, sambar deer, human, proboscis monkey, orang utan, clouded leopard	x	x	x	Luasong, Tawau, Sabah, Malaysia	5°06'N	117°30'E	170
Eleanor Slade & Li Yuen 2016/17, unpublished data	473	39	10	0.27	18.8	1.83	13.28	11.97	1.14	cattle, sun bear, elephant, bear cat, rhino, sambar deer, human, proboscis monkey, orang utan, clouded leopard	x	x	x	Luasong, Tawau, Sabah, Malaysia	5°06'N	117°30'E	170
Eleanor Slade & Li Yuen 2016/17, unpublished data	3255	41	9	0.12	18.11	1.61	12.25	10.76	1.13	cattle, sun bear, elephant, bear cat, rhino, sambar deer, human, proboscis monkey, orang utan, clouded leopard	x	x	x	Luasong, Tawau, Sabah, Malaysia	5°06'N	117°30'E	170
Eleanor Slade & Li Yuen 2016/17, unpublished data	2020	39	9	0.18	17.56	1.92	10.35	9.24	1.18	cattle, sun bear, elephant, bear cat, rhino, sambar deer, human, proboscis monkey, orang utan, clouded leopard	x	x	x	Luasong, Tawau, Sabah, Malaysia	5°06'N	117°30'E	170
Eleanor Slade & Li Yuen 2016/17, unpublished data	2056	40	9	0.21	16.59	1.8	11.64	10.38	0.94	cattle, sun bear, elephant, bear cat, rhino, sambar deer, human, proboscis monkey, orang utan, clouded leopard	x	x	x	Luasong, Tawau, Sabah, Malaysia	5°06'N	117°30'E	170
Eleanor Slade & Li Yuen 2016/17, unpublished data	528	40	9	0.22	21.05	2.1	14.45	12.83	1.37	cattle, sun bear, elephant, bear cat, rhino, sambar deer, human, proboscis monkey, orang utan, clouded leopard	x	x	x	Luasong, Tawau, Sabah, Malaysia	5°06'N	117°30'E	170
Whipple & Hoback 2012	7395	15	11	0.03	11.23	1.19	6.12	5.76	0.59	chimpanzee, human, pig, tiger, lion, cougar, zebra, waterbuck, moose, donkey, bison	x	x	x	Nebraska, USA	41°28'08.4"N	103°20'25.0"W	1300
Carpaneto et al. 2005	1708	20	2	0.65	10.37	1.83	3.32	3	0.46	sheep, dog	x	x	-	Pineto Urban Regional Park, Rome, Italy	41°55'17.10" N	12°25'45.82" E	100
Dormont et al. 2004	1367	28	2	0.21	20.41	1.54	15.94	14.23	1.33	cattle, horse	x	-	-	Saint-Martin de Londres, France	43°48' N	3°43' E	250
Errousi et al. 2004	4782	12	2	0.01	7.01	1.12	2.65	2.56	0.31	cattle, goat	x	-	-	Saint-Martin de Londres, France	43°48' N	3°43' E	250
Dormont et al. 2004	3089	21	2	0.29	11.27	1.36	7.18	6.67	0.64	cattle, horse	x	-	-	Le Rouet, France	43°49' N	3°50' E	250
Dormont et al. 2004	1216	16	2	0.08	12.62	0.96	9.37	8.85	0.66	cattle, horse	x	-	-	Mont Aigoual, France	44°08' N	3°34' E	1500
Dormont et al. 2007	1931	16	4	0.12	9.21	1.23	5.05	4.82	0.49	cattle, horse, deer, sheep	x	-	-	Mont Aigoual, France, pasture	44°08' N	3°34' E	1500
Dormont et al. 2007	3017	20	4	0.16	9.39	1.25	4.43	4.19	0.51	cattle, horse, deer, sheep	x	-	-	Mont Aigoual, France, pasture	44°08' N	3°34' E	1500
Dormont et al. 2007	385	5	4	0.04	4.23	0.62	2.69	2.64	0.17	cattle, horse, deer, sheep	x	-	-	Mont Aigoual, France, forest	44°08' N	3°34' E	1500
Dormont et al. 2007	1020	16	4	0.2	8.26	1.35	4.12	3.96	0.4	cattle, horse, deer, sheep	x	-	-	Mont Aigoual, France, forest	44°08' N	3°34' E	1500

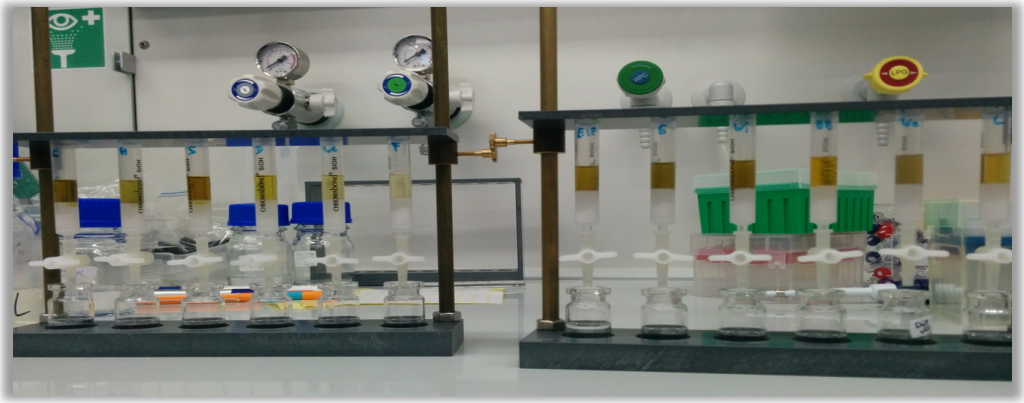
Table S4.1 (2/2): All datasets including geographic coordinates, the total number beetle species, and the degree of specialization.

Reference / year of data sampling	N beetles	N beetle species	N dung types	beetle-resource specificity (H2')	rarefied species richness	SD for rarefied species richness	Shannon diversity	rarefied Shannon diversity	SD for rarefied Shannon diversity	dung types	herbivore	omnivore	carnivore	sampling area	latitude	longitude	~ altitude (m a.s.l.)
Kessler et al. 1974	2429	14	2	0.26	10.18	1.27	5.92	5.59	0.54	cattle, sheep	x	-	-	South Dakota, USA	44°12'00.0"N	96°47'00.0"W	490
Jay-Robert et al. 2008	2014	26	2	0.27	13.34	1.74	7.12	6.44	0.74	sheep, deer	x	-	-	Cassagnas District, France	44°20' N	3°50' E	1000
Jay-Robert et al. 2008	1012	23	2	0.14	11.97	1.59	6.8	6.3	0.69	sheep, deer	x	-	-	Cassagnas District, France	44°20' N	3°50' E	1000
Jay-Robert et al. 2008	427	20	2	0.57	12.9	1.49	7.46	6.95	0.73	sheep, deer	x	-	-	Cassagnas District, France	44°20' N	3°50' E	1000
Jay-Robert et al. 2008	1738	21	2	0.59	7.81	1.59	3.05	2.84	0.38	sheep, deer	x	-	-	Cassagnas District, France	44°20' N	3°50' E	1000
Jay-Robert et al. 2008	743	17	2	0.42	8.39	1.46	2.74	2.56	0.3	sheep, deer	x	-	-	Cassagnas District, France	44°20' N	3°50' E	1000
Jay-Robert et al. 2008	1551	17	2	0.2	9.71	1.31	2.72	2.68	0.37	sheep, deer	x	-	-	Cassagnas District, France	44°20' N	3°50' E	1000
Ricou 1981, Diploma Thesis	952	13	2	0.15	10.15	0.85	7.25	6.91	0.51	sheep, cattle	x	-	-	Grandrieu, France	44°47'00.0"N	3°38'00.0"E	1800
Ricou 1981, Diploma Thesis	7885	13	2	0.01	8.14	1.3	3.89	3.71	0.4	sheep, cattle	x	-	-	Grandrieu, France	44°47'00.0"N	3°38'00.0"E	1800
Barbero et al. 1999	2244	10	4	0.19	9.38	0.68	7.35	6.97	0.43	cattle, horse, deer, boar	x	x	-	La Mandria Natural Park, Turin, Italy	45°10'4.72" N	7°34'42.30" E	300
Errousi et al. 2004	463	10	2	0.05	9.18	0.88	6	5.81	0.45	cattle, goat	x	-	-	Alps, France	45°19' N	7° E	1700
Wurmitzer et al. 2017 (Ilmitz) and unpublished data	7153	7	3	0.01	5.88	0.74	3.72	3.67	0.34	cow, donkey, horse	x	-	-	Ilmitz, Austria	47°46'14"N	16°45'54"E	115
Wurmitzer et al. 2017 (Ilmitz) and unpublished data	507	5	3	0.02	4.84	0.37	3.44	3.4	0.23	cow, donkey, horse	x	-	-	Ilmitz, Austria	47°46'14"N	16°45'54"E	115
Wurmitzer et al. 2017 (Ilmitz) and unpublished data	258	6	3	0.08	5.92	0.27	2.86	2.87	0.24	cow, donkey, horse	x	-	-	Ilmitz, Austria	47°46'14"N	16°45'54"E	115
Wurmitzer et al. 2017 (Ilmitz) and unpublished data	1143	9	3	0.01	7.98	0.59	5.48	5.29	0.43	cow, donkey, horse	x	-	-	Ilmitz, Austria	47°46'14"N	16°45'54"E	115
Wurmitzer et al. 2017 (Ilmitz) and unpublished data	3271	8	3	0.02	6.42	0.62	3.47	3.4	0.36	cow, donkey, horse	x	-	-	Ilmitz, Austria	47°46'14"N	16°45'54"E	115
Wurmitzer et al. 2017 (Ilmitz) and unpublished data	2272	9	3	0.05	7.98	0.64	4	3.88	0.46	cow, donkey, horse	x	-	-	Ilmitz, Austria	47°46'14"N	16°45'54"E	115
Frank et al. 2017b and unpublished data	171	34	6	0.37	9.5	0.56	5.79	5.7	0.34	cattle, horse, sheep, deer, wildboar, fox	x	x	-	Schwäbische Alb, Germany (Exploratories), grassland	48°25'32.0"N	9°24'06.6"E	700
Frank et al. 2017b and unpublished data	544	34	6	0.22	6.55	1.2	1.97	1.93	0.22	cattle, horse, sheep, deer, wildboar, fox	x	x	-	Schwäbische Alb, Germany (Exploratories), forest	48°25'32.0"N	9°24'06.6"E	700
Frank et al. 2017b and unpublished data	307	34	6	0.15	7.89	0.98	2.95	2.89	0.31	cattle, horse, sheep, deer, wildboar, fox	x	x	-	Schwäbische Alb, Germany (Exploratories), grassland	48°25'32.0"N	9°24'06.6"E	700
Frank et al. 2017b and unpublished data	1118	34	6	0.19	9.06	1.05	4.41	4.14	0.48	cattle, horse, sheep, deer, wildboar, fox	x	x	-	Schwäbische Alb, Germany (Exploratories), forest	48°25'32.0"N	9°24'06.6"E	700
Frank et al. 2017b and unpublished data	135	34	6	0.3	12.08	0.84	4.42	4.33	0.3	cattle, horse, sheep, deer, wildboar, fox	x	x	-	Schwäbische Alb, Germany (Exploratories), grassland	48°25'32.0"N	9°24'06.6"E	700
Frank et al. 2017b and unpublished data	553	34	6	0.16	5.25	0.7	2.62	2.54	0.25	cattle, horse, sheep, deer, wildboar, fox	x	x	-	Schwäbische Alb, Germany (Exploratories), forest	48°25'32.0"N	9°24'06.6"E	700
Frank et al. 2017b and unpublished data	199	34	12	0.22	4.52	0.87	1.8	1.75	0.12	cattle, horse, sheep, deer, wildboar, fox, elephant, wisent, elk, brownbear, lynx, wolf	x	x	x	Schwäbische Alb, Germany (Exploratories), forest	48°25'32.0"N	9°24'06.6"E	700
Cambefort & Walter 1991 and unpublished data	3108	72	3	0.82	29.95	2.66	26.07	20.21	1.92	human, elephant, buffalo	x	x	-	Tai forest, Ivory Coast	5°36'00.0"N	7°05'00.0"W	150
Nibaruta 1982, PhD Thesis	361	10	6	0.19	5.15	0.52	2.1	2.08	0.2	impala, bongo, nyala, eland, zebu, cattle	x	-	-	Belgium	50°34'11.9"N	5°33'17.2"E	270
Nibaruta 1982, PhD Thesis	436	10	6	0.27	6.37	0.66	4.05	3.92	0.28	impala, bongo, nyala, eland, zebu, cattle	x	-	-	Belgium	50°34'11.9"N	5°33'17.2"E	270
Frank et al. 2017b and unpublished data	194	34	6	0.39	7.19	0.75	2.85	2.81	0.24	cattle, horse, sheep, deer, wildboar, fox	x	x	-	Hainich, Germany (Exploratories), grassland	51°10'00.3"N	10°25'16.2"E	380
Frank et al. 2017b and unpublished data	378	34	6	0.17	7.85	1.05	2.72	2.66	0.3	cattle, horse, sheep, deer, wildboar, fox	x	x	-	Hainich, Germany (Exploratories), forest	51°10'00.3"N	10°25'16.2"E	380
Frank et al. 2017b and unpublished data	615	34	6	0.12	7.54	1.23	3.26	3.11	0.33	cattle, horse, sheep, deer, wildboar, fox	x	x	-	Hainich, Germany (Exploratories), grassland	51°10'00.3"N	10°25'16.2"E	380
Frank et al. 2017b and unpublished data	347	34	6	0.29	2.85	0.36	1.28	1.27	0.09	cattle, horse, sheep, deer, wildboar, fox	x	x	-	Hainich, Germany (Exploratories), forest	51°10'00.3"N	10°25'16.2"E	380
Frank et al. 2017b and unpublished data	319	34	6	0.21	11	1.08	7.13	6.83	0.58	cattle, horse, sheep, deer, wildboar, fox	x	x	-	Hainich, Germany (Exploratories), grassland	51°10'00.3"N	10°25'16.2"E	380
Frank et al. 2017b and unpublished data	1037	34	6	0.25	5.17	1	1.79	1.72	0.19	cattle, horse, sheep, deer, wildboar, fox	x	x	-	Hainich, Germany (Exploratories), forest	51°10'00.3"N	10°25'16.2"E	380
Frank et al. 2017b and unpublished data	284	34	12	0.24	8.64	1.59	2.4	2.3	0.27	cattle, horse, sheep, deer, wildboar, fox, elephant, wisent, elk, brownbear, lynx, wolf	x	x	x	Hainich, Germany (Exploratories), forest	51°10'00.3"N	10°25'16.2"E	380
Frank et al. 2017b and unpublished data	76	34	12	0.39	34	NA	2.87	2.87	NA	cattle, horse, sheep, deer, wildboar, fox, elephant, wisent, elk, brownbear, lynx, wolf	x	x	x	Hainich, Germany (Exploratories), grassland	51°10'00.3"N	10°25'16.2"E	380
Milotic et al. 2017	742	17	3	0.11	8.93	1.16	4.29	4.14	0.52	cattle, horse, sheep	x	-	-	Zwin, Belgium, autumn	51°21'21"N	3°20'41"E	5
Milotic et al. 2017	143	17	3	0.23	10.3	0.66	7.7	7.57	0.3	cattle, horse, sheep	x	-	-	Zwin, Belgium, summer	51°21'21"N	3°20'41"E	5
Milotic et al. 2017	6727	17	3	0.19	4.68	0.99	2.34	2.25	0.19	cattle, horse, sheep	x	-	-	Kalmthout, Belgium, autumn	51°24'54"N	4°25'10"E	20
Milotic et al. 2017	233	17	3	0.18	6.67	0.75	4.75	4.63	0.29	cattle, horse, sheep	x	-	-	Kalmthout, Belgium, summer	51°24'54"N	4°25'10"E	20
Frank et al. 2017b and unpublished data	4162	34	6	0.14	5.67	1.03	3.39	3.32	0.26	cattle, horse, sheep, deer, wildboar, fox	x	x	-	Schorfheide, Germany (Exploratories), forest	52°58'40.8"N	13°44'50.6"E	100
Frank et al. 2017b and unpublished data	85	34	6	0.28	34	NA	8.09	8.09	NA	cattle, horse, sheep, deer, wildboar, fox	x	x	-	Schorfheide, Germany (Exploratories), grassland	52°58'40.8"N	13°44'50.6"E	100
Frank et al. 2017b and unpublished data	1012	34	6	0.17	9.95	1.18	3.04	2.87	0.42	cattle, horse, sheep, deer, wildboar, fox	x	x	-	Schorfheide, Germany (Exploratories), forest	52°58'40.8"N	13°44'50.6"E	100
Frank et al. 2017b and unpublished data	932	34	6	0.12	5.91	1	1.82	1.75	0.2	cattle, horse, sheep, deer, wildboar, fox	x	x	-	Schorfheide, Germany (Exploratories), grassland	52°58'40.8"N	13°44'50.6"E	100
Frank et al. 2017b and unpublished data	6257	34	6	0.2	5.66	0.93	2.07	1.98	0.21	cattle, horse, sheep, deer, wildboar, fox	x	x	-	Schorfheide, Germany (Exploratories), forest	52°58'40.8"N	13°44'50.6"E	100
Frank et al. 2017b and unpublished data	605	34	12	0.23	8.44	1.31	3.05	2.95	0.38	cattle, horse, sheep, deer, wildboar, fox, elephant, wisent, elk, brownbear, lynx, wolf	x	x	x	Schorfheide, Germany (Exploratories), forest	52°58'40.8"N	13°44'50.6"E	100
Frank et al. 2017b and unpublished data	18	34	12	0.17	34	NA	5.3	5.3	NA	cattle, horse, sheep, deer, wildboar, fox, elephant, wisent, elk, brownbear, lynx, wolf	x	x	x	Schorfheide, Germany (Exploratories), grassland	52°58'40.8"N	13°44'50.6"E	100
Rainio 1966	10066	26	3	0.1	14.17	1.83	7.63	6.92	0.84	sheep, cattle, horse	x	-	-	Saari, Finland	60°44'03.4"N	25°25'07.7"E	80
Rainio 1966	6124	19	3	0.42	6.41	1.22	2.49	2.37	0.31	human, cattle, horse	x	x	-	Saari, Finland	60°44'03.4"N	25°25'07.7"E	80
Cambefort 1991	52220	123	2	0.48	28.27	2.93	24.55	18.21	2.2	human, cattle	x	x	-	Abokouamekro, Ivory Coast	7°04'00.0"N	5°05'00.0"W	175
Hewavithana et al. 2016	454	22	5	0.41	14.93	1.53	8.98	8.33	0.69	leopard, buffalo, cervid, elephant, bear	x	x	x	Wasgomuwa National Park, Sri Lanka	7°43'0"N	80°56'0"E	400

Chapter 5





5 Nutrient quality of vertebrate dung as a diet for dung beetles

Kevin Frank, Adrian Brückner, Andrea Hilpert, Michael Heethoff and Nico Blüthgen

5.1 Abstract

At the basis of a trophic web, coprophagous animals like dung beetles (Scarabaeoidea) utilize resources that may have advantages (easy gain and handling) as well as drawbacks (formerly processed food). Several studies have characterized the nutrients, e.g. C/N ratios and organic matter content, for specific types of dung. However, a comparative approach across dung types and feeding guilds of dung producers, and relationships between dung nutrients and preferences by coprophages, have been missing. Hence, we analysed water content, C/N ratio, amino acid, neutral lipid fatty acid, free fatty acids and sterol composition and concentrations in dung from 23 vertebrates, including carnivore, omnivore and herbivore species. Our analyses revealed significant differences among the three vertebrate feeding guilds for most nutritional parameters. Although formerly processed, dung grants sufficient amounts of essential nutrients for insects. We tested whether nutrients can explain the dung beetles' preferences in a field experiment, using 12 representative dung types in baits that were installed in 27 forests and 27 grasslands. Although consistent preferences for specific dung types were pronounced, the nutritional composition did not predict the variation in attractiveness of these dung diets, suggesting a primary role of dung volatiles irrespective of food quality.

5.2 Background

Heterotrophic organisms have to consume food to generate energy, grow and maintain metabolism(Lotka 1922), thus various strategies for detection, foraging and processing to exploit a wide range of diets has evolved(Schmidt-Nielsen 1997). Moreover the patchy distribution of manifold resources and its constant dynamics selected for differences in

feeding behaviours; ranging from opportunistic to highly specialised feeding(Begon et al. 2009). Some animals mainly use the metabolic trash of others (dung) to fulfil their energy requirements. Such coprophages (=“dung eaters”) substantially contribute to nutrient and energy flows in ecosystems (Nichols et al. 2008, Wu et al. 2011), since “in nature, nothing is wasted – not even waste” (Jones 2017).

One of the most common invertebrate coprophages are dung beetles (Scarabaeoidea); a cosmopolitan superfamily of insects which evolved a detritivorous life-style several million years ago (Hanski and Cambefort 1991, Chin and Gill 1996, Davis et al. 2002, Nikolajev and Dong 2010). Although the ancient detritivorous feeding behaviour (using all organic material, including dung, litter, humus and carcasses) still exists in many families of Scarabaeoidea (Halffter and Matthews 1966, Rembialkowska 1982), the increased occurrence of megafauna during the lower Jurassic has provided a new exploitable resource in large quantities, facilitating the evolution of dung beetles towards coprophagy (Cambefort 1991b). Dung beetles are often generalistic in their use of different types of faeces, although certain dung types are clearly more frequently utilized than others (Whipple and Hoback 2012b). Such preferences may be influenced and modified by the “host” animals’ diet (carnivore, herbivore, omnivore) which affects the nutrients, volatile organic compounds or odour intensities in their faeces (Halffter and Matthews 1966, Gittings and Giller 1998, Schmitt et al. 2004, Dormont et al. 2007, Scholtz et al. 2009, Whipple and Hoback 2012b). Dung nutrients are of particular importance in dung beetle development, e.g. their body size or the length of the male’s horns (Emlen 1997, Moczek and Emlen 1999). Dung itself consists of metabolic waste products and undigested remains of the original food. However, also other food sources like humus, fungi and carrion are used by dung beetles (Hanski and Cambefort 1991), thus questioning dung as the only trophic resource for dung beetles which can supplement them with all mandatory nutrients. Furthermore, endosymbiotic bacteria associated with dung beetles may facilitate the digestion of dung and could foster a well-balanced nutritional supplementation (Halffter and Matthews 1971, Rougon et al. 1990, Estes et al. 2013) as regularly found in others insects (Douglas 2009, Kaltenpoth 2009, Gibson and Hunter 2010). Several studies analysed C/N ratios, organic matter contents, amino acids as well as further components (recently reviewed by Holter (2016)) and addressed the high variability of composition and nutritional values among dung types and feeding guilds (Whipple and Hoback 2012b). However, a comparative approach analysing the nutrients, dung type preferences and nutrient-preference-relationships in a broad variety of dung types from different species has not been conducted so far.

Hence, we address the following questions; i) does the nutritional composition differ among the dung from different feeding guilds of vertebrates; ii) can this dung potentially supply all essential macronutrients for insects like dung beetles; ii) does the dung differ in its attractiveness to dung beetles, and if; iii) do preferences correspond to nutritional composition? We analysed C/N ratios, amino and fatty acids, sterol and water contents of dung from 23 vertebrate species (7 carnivores, 6 omnivores and 10 herbivores) using gas-chromatography (GC), gas chromatography/mass spectrometry (GC/MS) and ion-exchange chromatography (IEC). Furthermore, we used 12 (out of the 23) dung types for a field experiment to compare the attractiveness of dung from different feeding guilds to dung beetles. We show that i) the nutritional composition of dung differed among feeding guilds, although almost all essential macronutrients were found in all samples; ii) dung beetles showed significant dung type preferences; and iii) preferences did not correspond to nutritional parameters.

5.3 **Methods**

Dung sampling and processing

We used 23 different dung types of carnivorous (otter, lynx, mink, raven, snowy owl, wolf, wild cat), omnivorous (chicken, wild boar, brown bear, fox, gerbil, raccoon) and herbivorous (cow, donkey, deer, elephant, elk, goat, horse, rabbit, sheep, wisent) species, which we collected at two organic farms, wildlife parks/zoos and private stocks around Darmstadt (detailed information on origin and diet of each species are provided in the supplementary material; Table S5.2 and supplementary methods S5.1). Furthermore, all animals involved in this study had not faced any veterinarian treatment for several weeks before dung collection. After collecting fresh samples (i.e. droppings from the collection day) in a sufficient amount, the dung was filled in a tea bag (for dung baits used in the field), subsequently transferred into a freezer bag, sealed and labelled. A part of the sampled dung was further processed for chemical analysis (see below). All samples were stored in a freezer at -20 °C until further use.

Field sampling and study site

For dung beetle field samplings we used pitfall traps equipped with the dung baits of 12 different representative subsamples (i.e. dung available in sufficient amounts) collected form mammal species, namely: wolf, lynx, fox, brown bear, wild boar, cow, horse, sheep,

deer, elephant, elk and wisent (2 carnivores, 3 omnivores and 7 herbivores). The traps were set up randomized on a transect, in a total of 54 experimental sites (27 in forests, 27 in grasslands) in three regions of Germany within the Biodiversity Exploratories' framework (see supplementary methods S5.1). All field samplings were performed between 29th June and 17th July 2015. Pitfall traps were collected after 48 hours, trapped beetles were labelled (date, site-ID, dung type) and stored in a freezer at -20 °C. Dung beetles were identified to species level using the keys of Freude et al. (1969), Bunalski (1999) and Rössner (2012), and confirmed by taxonomic experts (see Acknowledgements).

Water content and dry weight

Water content of each dung was determined with a microbalance (Mettler Toledo, XS3DU, Columbus OH, USA; readability 0.1 µg and 1 µg repeatability) two times with three replicates (20 mg, 50 mg and 100 mg) per dung; (a) the initial fresh mass of the dung prior to drying and (b) its dry mass after the dung has been dried until weight constancy in an oven at 60 °C for at least 4 days. For the determination of the dry weight of the bird faeces (raven and snowy owl), which were partly absorbed in sand, the actual dry organic matter was determined by fully annealing the dung samples with a Bunsen burner and back-weighing of the ignition residues. Linear regression formulas for the calculation of each dung's dry weight are given in the supplementary information (List S5.1).

Lipid and sterol analyses

Total neutral lipids (hereafter, neutral lipid fatty acids = NLFAs) were extracted from the fresh dung samples (40 - 50 mg of fresh weight) using 1 ml of a chloroform:methanol-mixture, 2:1 (V/V) according to (Folch et al. 1957) over a period of 24 h. Afterwards two replicate extracts were purified and separated according to the methods described by (Frostegard et al. 1991) and (Tserng and Griffin 2003) to fractionate neutral lipid fatty acids (NLFAs) and free fatty acids (FFAs), respectively (for detailed procedure see supplementary methods S1). After the samples had been fractionated by column chromatography, they were finally measured with a QP2010 Ultra GC/MS (Shimadzu, Duisburg, Germany). The gas chromatograph (GC) was equipped with a ZB-5MS fused silica capillary column (30 m x 0.25 mm ID, df= 0.25 µm) from Phenomenex (Aschaffenburg, Germany). 1 µl sample aliquots were injected by using an AOC-20i autosampler-system (Shimadzu, Duisburg, Germany) into a PTV-split/splitless-injector (Optic 4, ATAS GL, Eindhoven, Netherlands), which operated in splitless-mode. Injection-temperature was programmed from initial 70 °C up to 300 °C and

then an isothermal hold for 59 minutes. Hydrogen was used as carrier-gas with a constant flow rate of 1.5 ml/min. The temperature of the GC oven was raised from initial 60°C for 1 min, to 150°C with a heating-rate of 15°C/min, to 260°C with a heating-rate of 3°C/min, to 320°C with a heating-rate of 10°C/min and then an isothermal hold at 320°C for 10 min. Electron ionization mass spectra were recorded at 70 eV from *m/z* 40 to 650. The ion source of the mass spectrometer and the transfer line were kept at 250°C. FAMEs were identified based on their retention indices (Stein 2015) and *m/z* fragmentation patters as well as by comparison with the FAME and BAME analytical standards (Sigma-Aldrich, St. Lois, USA). The configurations of the double bonds were not specifically determined. The amount of fatty acids (i.e. NLFAs and FFAs) [µg] was standardized using the dry weight [mg] calculated form the initial fresh weight of the sample.

Sterols were quantified based on the peak area of detected compounds relative to the constant amount of the internal standard (220 ng/µl nonadecanoic acid) expressed in [%] of this standard, because we did not determine the response factor of the sterols to the internal standard. Cholesterol was the only sterol that was identified based on its *m/z* fragmentation [as cholesteryl methyl ether: 400 (M^+, 60); 385 (24); 368 (100); 353 (59), 329 (31), 301 (25), 275 (37), 213 (26), 145 (42), 107 (50), 81 (46), 69 (27), 55 (41)], for the other sterols we just checked for correct substance class assignment (as sterols) based on their mass spectra. The amounts of sterols [% Std.] and cholesterol [% Std.] were standardized using the dry weight [mg] calculated form the initial fresh weight of the sample. The cholesterol/sterol ratio [%] was calculated based on both values.

Amino acid analysis

For analysis of the amino acids (free amino acids and protein-bounded), 5 mg (± 0.1 mg) dried dung was diluted in 200 µL of hydrochloric acid (6 mol/l) and boiled for four hours at 100°C, processed (for detailed procedure see supplementary methods S1) and finally measured as described in(Leonhardt and Blüthgen 2012) with an ion exchange chromatograph with ninhydrin post-column derivatization (Biochrom 20+, Amino Acid Analyzer, Cambridge, UK). A standard amino acid mixture (Laborservice Onken GmbH, Gründau, Germany) was used as external standard. The amount of total amino acids [µg] was standardized using the dry weight [mg] of the initial sample. Note that this acidic chemical extraction decays asparagine, glutamine and tryptophan.

C/N analysis

Dried dung samples were mixed with hydrochloric acid (HCl; approx. 0.05 mol/l) to remove the inorganic carbonate as CO_2. Samples were subsequently dried again and weighed into tin capsules (6±1 mg dry weight). Total organic carbon and nitrogen contents were measured by an elemental analyser (EA 1108 Elemental Analyser, Carlo Erba, Milan, Italy). Acetanilide (Merck, Darmstadt, Germany) was used for internal calibration.

Data processing and statistical analyses

Preferences of dung beetles across different dung were analysed on three different levels: i) The number of dung beetles collected per plot (dependent variable) on a certain dung type (independent variable) was analysed using Friedman test with plot ID as blocking factor. ii) The number of dung beetles collected on each plot/number of traps (dependent variable) on dung of the three different feeding guilds (independent variable) was analysed using Friedman test with plot ID as blocking factor, as well. iii) The proportional abundance [%] of each beetle species among the dung from the three different feeding guilds was visualized as bipartite network and analysed using H_2' (Blüthgen et al. 2006) as a measurement for the overall specialization of the compositional dung type – beetle species network. The network analysis was performed with the R package "bipartite" (Dormann et al. 2009).

We statistically analysed the C/N ratio, amount of amino/fatty acids [µg/mg], the water content [%] and the amounts of sterols [% Std./mg] of dung types (carnivore, omnivore, herbivore) using ANOVA and TukeyHSD post-hoc test. We checked for the normal distribution of the residuals and the homogeneity of variance prior to the analyses and transformed the data if necessary (C/N ratio, the amount of NLFAs s [µg/mg] were log-transformed, the amounts of sterols [% Std./mg] were log(x+1)-transformed, the ratio of FFA/total lipids [%] was arcsine square root transformed whereas the amount of free fatty acids [µg/mg] and amino acids [µg/mg] were square root transformed and 4^{th}-square root transformed, respectively). To compare the cholesterol/ sterol ratio [%] and the amount of FFAs [µg/mg] across dung types we used Kruskal-Wallis tests with subsequent pairwise U-tests corrected for false discovery rate (Benjamini and Hochberg 1995) in multiple comparisons.

Compositional data of amino- and NLFA profiles were analysed using discriminant analysis of principal components (DAPC) and PERMANOVA/PERMDISP as implemented in the R packages "adegenet" (Jombart 2008) and "vegan" (Oksanen et al. 2007),

respectively. DAPC is a powerful method to discriminate *a priori* assigned groups in a multivariate ordination of chemical compositional data (Brückner and Heethoff 2017). It transforms the original data by PCA prior to the discriminant analysis (DA) and therefore values become uncorrelated. We retained 7 (for amino acids) and 5 (for NLFAs) PC-axes based on their Eigenvalues and the explained variance. We further used PERMANOVA (Anderson 2001) with Bray-Curtis dissimilarities (Bray and Curtis 1957) to test if overall composition of either amino acids (all amino acids and essential amino acids) or NLFAs differed across dung types. In case of significant PERMANOVA, we used PERMDISP (Anderson 2006) to distinguish between location/dispersion effects (see (Anderson et al. 2008)and Brückner and Heethoff (2017) for details) and to test whether the compositional stability of nutrients differed among dung types. All statistical analyses were performed with R version 3.3.1 “Bug in Your Hair” (R Core Team 2016). Finally, we correlated the total number of dung beetles (i.e. pooled from all plots) trapped on the respective dung type to different nutritional parameters of the dungs (means of C/N ratio, amounts of all/essential amino acids, NLFAs, FFAs and sterols as well as cholesterol/sterol ratio and water content) using Spearman's rank correlation in PAST 3 (Hammer et al. 2001).

5.4 **Results**

Nutritional analyses

Nutrients in vertebrate dung samples (see summarized in Table 5.1) differed significantly across the three feeding guilds (ANOVA; C/N ratio: $F_{2,18}$= 26.5, p< 0.001, Fig. 5.1a; amino acids: $F_{2,20}$= 3.7, p= 0.044, Fig. 5.1b; NLFAs: $F_{2,20}$= 4.3, p= 0.028, Fig. 5.1c), except for water content ($F_{2,20}$= 3.1, p= 0.065, Fig. 5.1d) and FFAs (Kruskal-Wallis test; χ^2= 2.6, d_f= 2, n= 21, p= 0.267, Fig. 5.2a).

Table 5.1: Nutrient parameters of vertebrate dung samples for the three feeding guilds; carnivores (n = 7), omnivores (n = 6) and herbivores (n = 10). The mean (±standard deviation) of the different nutrient parameters are shown for each feeding guild. NLFAs= neutral lipid fatty acids, FFAs= free fatty acids; – no measurement; § = double bond position not further determined

	C/N ratio	Amino	NLFAs	FFAs	FFAs/total	Water	Sterols	Cholesterol/
Carnivores								
lynx	5.4	166	105	33	24	62	27	47
mink	5.0	104	71	2	3	31	55	31
otter	5.4	47	49	8	14	78	1	77
raven	-	10	60	-	-	78	2	58
snowy owl	-	12	39	-	-	87	11	74
wildcat	6.1	131	21	2	9	48	30	0.3
wolf	11.3	20	58	2	3	39	1	74
mean	6.6±2.4	70±59	58±24	9±12	11±8	61±20	18±19	52±26
Omnivores								
brown bear	16.3	34	47	8	15	78	11	2
chicken	12.1	22	6	3	33	55	0.1	69
fox	6.6	40	26	3	10	48	2	21
gerbil	13.2	34	11	2	15	7	14	1
raccoon	11.1	90	65	2	3	59	3	35
wild boar	12.5	21	45	3	6	35	2	5
mean	11.9±2.9	40±23	33±21	4±2	14±10	47±21	5±5	22±24
Herbivores								
cow	21.3	21	17	8	32	84	7	19
deer	13.9	30	38	6	14	63	2	4
donkey	59.2	4	27	5	16	76	3	1
elephant	23.3	8	51	8	14	77	3	2
elk	24.3	14	12	6	33	71	26	0.2
goat	25.7	8	8	2	20	42	6	2
horse	36.6	9	23	5	18	76	2	4
rabbit	23.4	44	8	4	33	69	2	1
sheep	18.7	32	26	6	19	70	17	1
wisent	21.7	19	23	8	26	85	4	6
mean	26.8±12.1	19±12	23±13	6±2	21±8	71±12	7±7	4±5

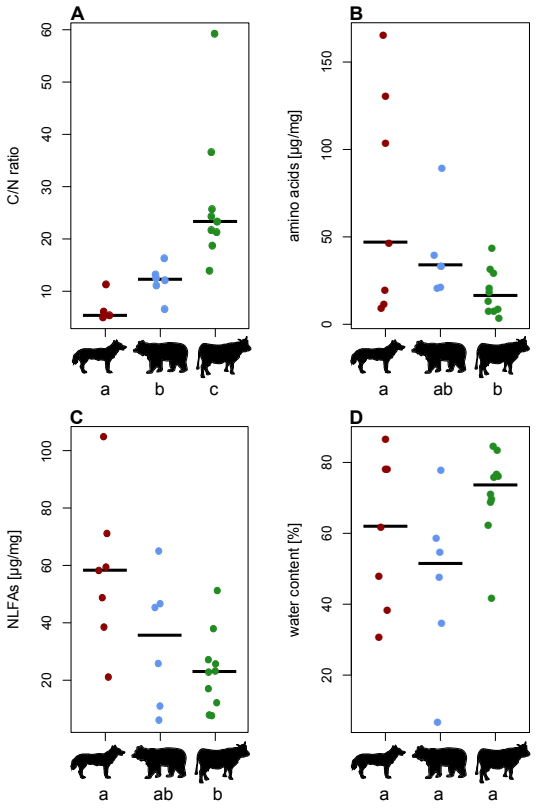


Figure 5.1: Nutritional values (C/N ratio (A), amino acids (B), neutral lipid fatty acids - NLFAs (C) and water content (D)) for carnivore, omnivore and herbivore dung (left to right). Lines within each feeding guild represent the median. Significanct differences among groups are given in different letters for each panel.

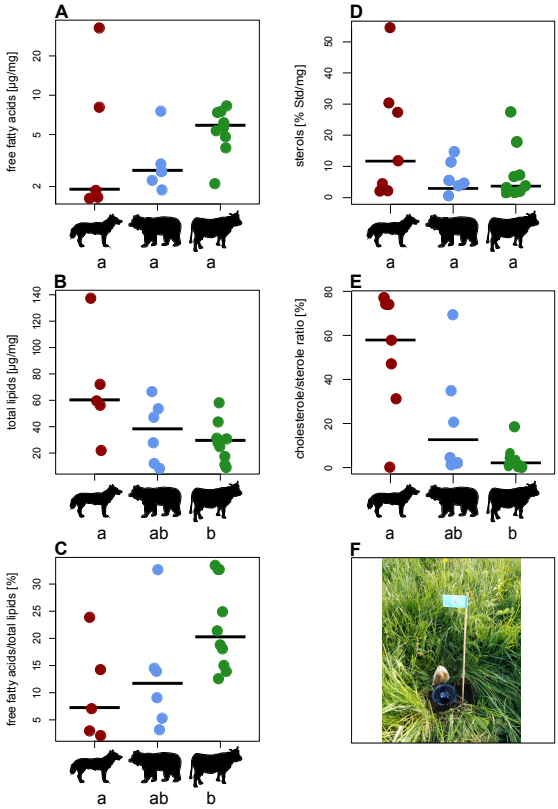


Figure 5.2: Nutritional values (free fatty acids (A), total lipids (B), free fatty acids/total lipids ratio (C), sterols (D) and cholesterol/sterol ratio (E)) for carnivore, omnivore and herbivore dung (left to right). Lines within each feeding guild represent the median. Significanct differences among groups are given in different letters for each panel. (F) shows a pitfall trap with dung bait for dung beetle sampling.

However, the total lipid content (NLFAs and FFAs combined) showed differences among the feeding guilds (ANOVA; $F_{2,18}$= 3.9, p= 0.039, Fig. 5.2b) and the amounts of FFAs and NLFAs were not correlated (Spearman´s rank: ρ_s= 0.14, p= 0.55). Also for the FFA/total lipid ratio and cholesterol/sterol ratio we found significant differences among the feeding guilds (FFAs/total lipid ratio: ANOVA; $F_{2,18}$= 4.4, p= 0.028, Fig. 5.2c; cholesterol/sterol ratio: Kruskal-Wallis test; χ^2= 8.2, d_f= 2, n= 23, p= 0.017, Fig. 5.2e), while sterol amounts were similar (ANOVA; $F_{2,20}$= 0.9, p= 0.44, Fig. 5.2d). The amino acids differed in relative composition between the feeding guilds (PERMANOVA; all amino acids: pseudo$F_{2,20}$= 2.7, R^2= 0.22, p= 0.013, Fig. 5.3a; only essential amino acids: pseudo$F_{2,20}$= 2.2, R^2= 0.18, p= 0.039) but not in overall dispersion across species within each guild (PERMDISP; all amino acids: $F_{2,20}$= 1.5, p= 0.25, Fig. 5.3a, Fig. S5.II, Table 5.2; only essential amino acids:

$F_{2,20}$= 1.1, p= 0.36), whereas NLFAs differed both in composition and dispersion (PERMANOVA; pseudo$F_{2,20}$= 4.8, R^2= 0.32, p< 0.001; PERMDISP: $F_{2,20}$= 5.0; p= 0.017, pairwise PERMDISP herbivore vs. omnivore p= 0.008; Fig. 5.3b, Fig. S5.I, Tab 5.3).

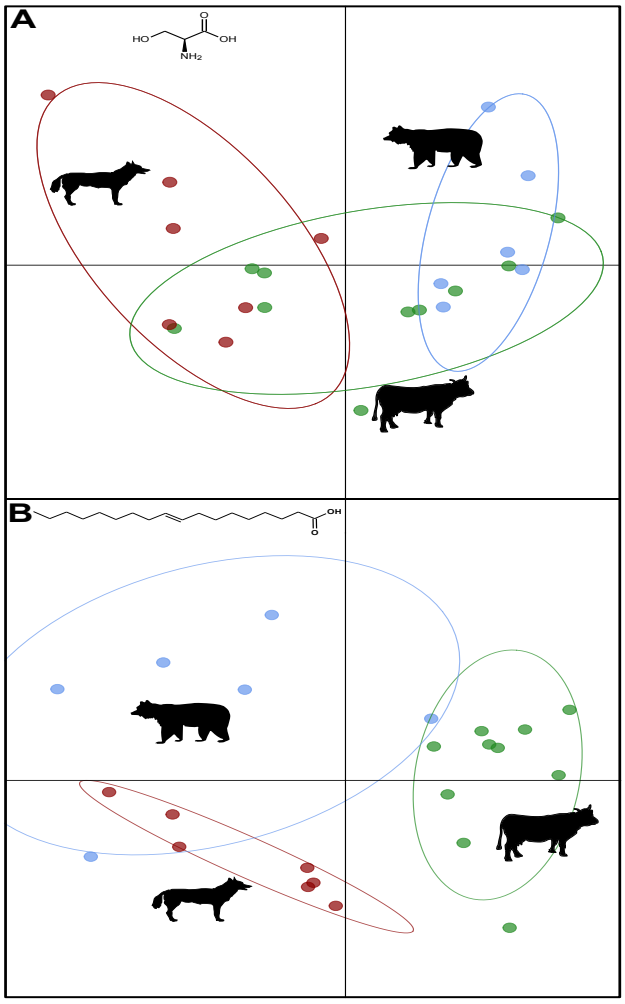


Figure 5.3: Discriminat analysis of principal components (DACP) for amino acids (A) and neutral lipid fatty acids - NLFAs (B). Feeding guilds are clustered in red for carnivore, in blue for omnivore and in green for herbivore dung.

In summary (see Table 5.1), carnivore dung types provided higher nutritional values (highest mean values for amino and fatty acids, sterols and cholesterol, lowest C/N ratio). Omnivore dung types provided intermediate nutritional value throughout the analyses.

Table 5.2: Free and protein-bounded amino acids [in %] for each dung type, sorted by feeding guild (type: C = carnivore, O = omnivore, H = herbivore).

species	type	amino acid [%]																
		OH-Pro	Thr	Ser	Glu	Pro	Gly	Ala	Val	Cys	Met	Ile	Leu	Tyr	Phe	Lys	His	Arg
lynx	C	1.8	5.4	9.5	11.7	8.5	18.3	14.1	5.3	1.2	1.0	2.3	7.1	1.8	2.6	5.3	1.5	2.7
mink	C	-	5.9	10.9	9.6	7.6	16.4	13.1	3.8	-	-	2.7	14.0	3.1	2.9	6.2	0.9	3.0
otter	C	-	9.6	13.3	10.1	9.9	16.5	14.7	3.7	-	-	1.2	9.7	2.1	2.1	4.2	1.0	1.8
raven	C	-	5.9	9.8	13.1	8.4	18.4	13.1	3.7	0.5	1.3	2.6	8.3	1.9	2.0	6.1	2.2	2.7
snowy owl	C	-	5.1	13.6	12.1	9.6	15.5	9.8	4.2	2.6	1.2	3.0	7.9	2.3	3.5	4.4	1.5	3.6
wildcat	C	3.7	4.4	8.4	12.3	10.2	23.1	11.3	3.3	0.4	1.2	1.9	6.0	1.4	2.2	5.4	2.1	2.7
wolf	C	1.1	6.0	10.3	12.3	8.6	17.2	11.4	5.6	0.9	1.3	2.7	7.5	1.8	2.9	5.4	2.0	3.0
brown bear	O	0.5	5.6	8.3	12.5	6.6	16.1	16.3	5.8	-	1.7	2.8	8.0	2.0	3.1	7.3	1.5	1.9
chicken	O	-	5.1	7.0	9.0	9.8	19.9	17.5	2.3	-	0.5	2.5	10.7	1.5	2.8	8.6	0.7	2.2
fox	O	0.7	4.6	8.0	10.6	7.0	20.7	16.4	3.9	-	1.2	2.8	8.2	1.5	2.9	8.0	1.7	1.7
gerbil	O	-	5.4	8.0	11.7	7.1	17.4	17.8	4.2	-	1.4	2.8	8.6	1.9	2.9	7.5	1.5	2.0
raccoon	O	-	5.5	7.4	11.6	7.1	19.9	18.9	2.0	-	-	2.2	10.7	1.6	2.5	8.2	0.7	1.6
wild boar	O	1.2	5.0	8.2	13.2	6.3	17.6	17.7	3.7	-	1.5	2.6	9.9	1.6	3.0	5.3	1.4	1.8
cow	H	0.2	5.2	7.8	11.1	8.1	17.4	16.1	3.6	-	1.2	2.7	10.1	1.9	3.2	8.0	1.3	2.1
deer	H	-	5.7	8.3	12.9	6.9	15.8	16.7	4.1	-	1.6	2.7	9.1	2.0	3.3	7.2	1.4	2.1
donkey	H	-	4.8	10.8	12.8	7.5	16.2	10.9	4.3	3.5	1.3	2.9	8.2	2.9	3.0	6.1	1.4	3.3
elephant	H	0.1	4.4	14.8	9.9	10.3	16.8	9.3	4.7	2.9	0.9	3.1	7.6	3.1	4.0	3.1	1.1	3.8
elk	H	-	4.6	7.3	11.6	7.5	19.6	17.3	2.7	-	0.8	2.3	9.8	1.6	2.9	8.3	1.5	1.9
goat	H	2.6	3.5	6.2	12.2	8.1	19.8	14.8	4.9	0.2	1.0	2.9	7.6	1.8	3.0	7.6	1.3	2.5
horse	H	1.6	5.3	8.0	12.5	8.8	18.3	13.9	6.1	0.2	1.1	2.6	7.2	1.6	2.7	6.3	1.6	2.1
rabbit	H	-	6.4	7.6	9.9	7.5	17.5	20.1	3.1	-	1.0	2.3	9.4	1.7	2.5	8.0	1.4	1.8
sheep	H	0.1	6.1	10.3	13.3	8.6	17.2	11.7	4.2	0.9	1.5	2.7	8.4	2.0	2.4	5.3	2.3	3.0
wisent	H	1.8	4.8	8.3	13.0	9.3	20.5	13.2	3.2	0.1	1.3	2.2	7.4	1.4	2.7	6.2	2.1	2.5

Table 5.3: Neutral lipid fatty acids [in %] derived from dietary saturated, mono- and polyunsaturated fats for each dung type, sorted by feeding guild (type: C = carnivore, O = omnivore, H = herbivore).

		fatty acids derived from dietary fats [%]																				
		saturated													monounsaturated				polyunsaturated			
species	type	C10	C12	C14	C15	C16	C17	C18	C20	C22	C24	C26	C28	C16:1 Δ7	C16:1 Δ9	C18:1 Δ9	C18:1 Δ10	C18:1 Δ11	C18:2 Δ9,12	C18:2	C20:4 Δ5,8,11,14	C20:3 Δ8,11,14
snowy owl	C	0.6	0.9	3.0	1.2	43.2	0.3	41.1	-	-	-	-	-	-	1.0	5.5	0.8	-	2.4	-	-	-
lynx	C	0.2	0.1	1.8	0.7	39.8	1.2	44.7	0.3	0.2	0.2	-	-	-	0.3	8.4	0.6	0.7	0.6	0.1	0.1	-
mink	C	0.1	-	0.2	-	39.3	0.3	17.9	0.3	0.1	0.1	-	-	0.1	0.2	40.7	-	-	0.7	0.1	-	-
otter	C	0.2	0.2	1.4	0.3	55.5	0.3	18.6	-	-	-	-	-	-	1.0	11.5	1.1	-	7.8	2.2	-	-
raven	C	0.3	0.6	1.7	0.4	35.5	0.8	42.6	0.5	-	-	-	-	-	0.8	9.7	1.5	-	4.1	1.0	0.6	-
wildcat	C	-	0.1	0.7	2.6	71.2	0.4	20.3	0.2	0.3	0.3	-	-	0.2	0.2	0.7	0.8	1.2	0.7	0.1	-	0.1
wolf	C	0.1	0.1	2.0	0.8	47.3	1.7	40.1	0.3	0.1	0.1	-	-	0.1	0.4	4.3	0.8	1.1	0.7	0.1	-	0.1
rabbit	H	0.1	0.3	1.0	1.3	35.5	0.4	28.4	0.4	0.5	0.3	0.3	0.3	-	0.8	1.9	0.5	-	5.7	22.5	-	-
elk	H	0.4	1.0	3.9	4.3	33.7	3.8	38.8	0.9	0.9	1.1	0.8	1.1	-	-	2.4	3.6	-	2.1	0.6	-	-
elephant	H	0.1	0.4	2.0	2.1	20.3	0.7	58.4	0.8	0.6	0.3	0.2	0.2	-	-	1.8	1.6	8.4	0.9	0.9	-	-
donkey	H	0.1	0.6	7.0	7.4	33.4	2.2	38.1	0.5	0.7	0.6	0.9	0.5	-	0.1	1.1	3.3	-	0.9	0.3	-	-
cow	H	0.5	0.9	3.7	2.3	32.7	2.3	46.8	0.4	0.5	0.6	0.4	0.5	-	0.1	1.9	1.9	2.6	0.6	-	-	-
horse	H	0.2	1.0	5.7	6.2	36.2	1.7	37.2	0.8	0.6	0.6	0.5	0.4	-	-	1.7	5.2	-	0.8	-	-	-
deer	H	0.2	0.4	1.7	1.9	22.6	1.5	63.6	0.6	0.4	0.4	0.5	0.0	-	-	1.6	3.1	-	0.9	0.2	-	-
sheep	H	0.2	0.9	2.9	4.0	27.5	2.4	48.0	0.7	0.5	1.2	1.6	0.4	-	-	1.6	2.9	3.2	0.7	0.2	-	-
wisent	H	0.3	0.8	2.5	2.0	26.4	1.8	55.7	0.6	0.5	0.5	1.5	0.5	-	-	1.6	0.7	2.5	0.9	0.2	-	-
goat	H	0.5	1.6	4.8	3.8	33.7	2.9	37.1	1.3	1.1	1.0	2.9	2.3	-	-	2.1	0.7	-	2.4	0.7	-	-
brown bear	O	0.4	1.6	4.7	3.3	33.3	2.8	37.0	1.2	0.9	0.8	2.2	1.4	-	-	2.5	1.3	0.7	4.1	0.5	-	-
chicken	O	1.2	1.2	1.5	0.1	81.8	0.2	9.7	0.1	0.1	0.1	-	-	-	-	2.4	0.3	-	1.1	0.1	-	-
fox	O	0.1	0.3	2.0	0.7	41.9	0.4	15.8	0.3	0.3	0.3	-	-	-	4.8	24.7	3.9	1.6	2.2	0.8	-	0.1
gerbil	O	-	0.3	1.0	0.9	32.1	0.2	14.6	0.8	0.3	0.2	0.2	-	-	0.1	36.3	0.4	-	11.6	0.9	-	-
racoon	O	-	-	0.1	-	12.3	-	4.8	0.7	1.6	0.4	-	-	-	-	77.4	-	-	1.8	0.4	0.2	0.2
wild boar	O	0.1	0.2	1.4	2.3	27.7	1.1	39.4	0.6	0.3	0.2	0.1	-	-	0.2	8.5	1.5	10.7	4.3	1.5	-	-

Dung type preference

We sampled a total of 1191 individuals from 23 dung beetle species in 40 sites; in 14 out of 54 sites no dung beetles were trapped. Overall, dung beetles were attracted to all 12 dung types offered (Fig. 5.4c). Species-specific preferences of the beetles towards offered dung types, showed significant differences for the "host animals" (Friedman test: χ^2= 62.1, d_f= 11; n= 648, p< 0.0001, Fig. 5.4a), but not for corresponding feeding guilds (Friedman test: χ^2= 2.8, d_f= 2, n= 162, p= 0.25, Fig. 5.4b). Different beetle species had relatively similar preferences and showed no clear species partitioning across dung types, hence there was only a relatively low degree of complementary specialization in the dung type – beetle network (H_2'= 0.30).

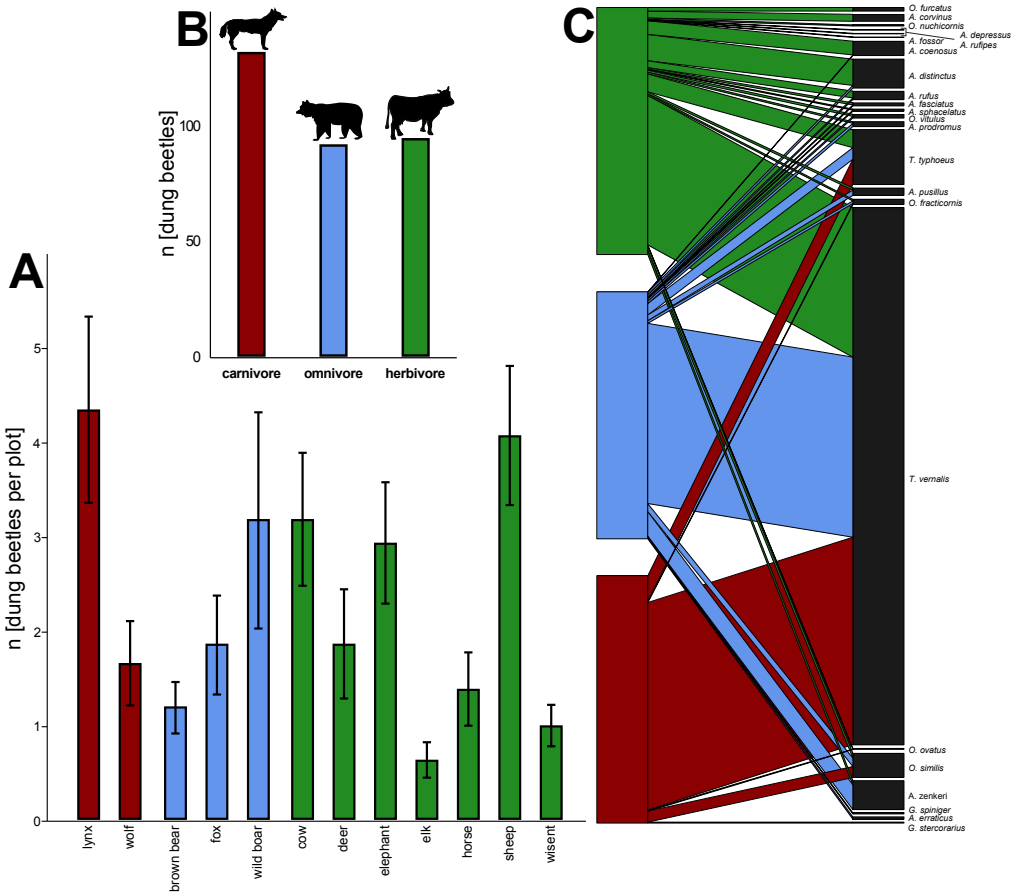


Figure 5.4: Overview of trapped beetles for a subset of representative dung types, used in a field sampling. (A) shows the mean number of trapped beetles for each dung type (red = carnivore, blue = omnivore, green = herbivore), while (B) shows the total number of trapped beetles per feeding guild. (C) highlights in a resource - beetle interaction network the attraction for sampled dung beetle species towards dung of the feeding guilds (red = carnivore, blue = omnivore, green = herbivore).

Correlation between dung nutrients and attractiveness for dung beetles

Overall, there was no correlation between dung beetle abundances and any of the nutrient parameters for all 12 dung types (Spearman´s rank: C/N ratio: ρ_s= -0.48, p= 0.11; all amino acids: ρ_s= 0.46, p= 0.14; essential amino acids: ρ_s= 0.42, p= 0.17; NLFAs: ρ_s= 0.40, p= 0.20; FFAs: ρ_s= 0.16, p= 0.62; water content: ρ_s= -0.36, p= 0.24; sterols: ρ_s= 0.15, p= 0.55, cholesterol/sterol ratio: ρ_s= 0.26, p= 0.41).

5.5 **Discussion**

Dung beetles strongly depend on a resource that is scarce and patchy in occurrence. Yet, dung is immobile as well as chemically and mechanically undefended, which makes it an easily acquirable and valuable source of energy.

Adult dung beetles are attracted to many different dung types, regardless on which dung they have fed and grown as a larva (Hanski and Cambefort 1991, Dormont et al. 2010, Whipple and Hoback 2012b). Furthermore, especially in the tropics, some of these beetles became highly specialized in resource usage (Larsen et al. 2006). Our field experiment demonstrated a generalized usage of all offered dung types, but also a significant difference in the quantity of beetles attracted across dung types (Fig. 5.4a-c). Generally, higher amounts of dung attract more dung beetles (Errouissi et al. 2004), but since we used equal amounts of dung for the baits (approx. 35 g each) there must be alternative explanations for their preferences, for which the amount and composition of volatile organic compounds must play a key role (Schmitt et al. 2004, Wurmitzer et al. 2017)given that the beetles had no contact to the dung in our experiment (see Fig. 5.2f). For instance, indole and skatole, two weak/moderate attractors (Wurmitzer et al. 2017) are molecules derived from the decomposition of the amino acid tryptophan, whereas phenolic compounds (e.g., phenol, p-cresol, p-ethyl phenol) are derivatives of phenylalanine and tyrosine. Also, fatty acids and fatty acid derived compounds like butyric, unspecific butanones and butanoles or ethyl-/butyl-esters are present in various dungs (Dormont et al. 2010, Stavert et al. 2014). Hence, dung odour bouquets as proximate cues may also include intrinsic information on their ultimate cause, i.e. dung nutrients (especially amino- and fatty acids) which are converted and rearranged to volatile organic compounds. Accordingly, besides the attractive function of dung volatiles (Dormont et al. 2010, Stavert et al. 2014, Wurmitzer et al. 2017), these compounds may also serve as “nutritional cues” for dung beetles. Therefore we asked whether

the beetles' preference matched the differences in nutrient quality of dung. Although beetles used all dung types, some were strongly preferred over others, and the most preferred ones occurred across the feeding guilds (e.g. lynx, wild boar and sheep) – hence with strong differences in quality. Nutritional composition was no significant predictor to explain the beetle's preference. Hence, volatiles are most probably no nutritional cues. Yet, volatile organic compounds only recently receive growing attention in dung beetle research (Schmitt et al. 2004, Dormont et al. 2007, Dormont et al. 2010, Mansourian et al. 2016, Wurmitzer et al. 2017) and more in depth analyse may help to unravel the beetles' resource choices linked to dung nutrients (Stavert et al. 2014).

In general animal droppings vary in nutrient amounts, even within a species or feeding guild (Hanski 1987). Whereas higher nutrient concentrations are generally beneficial, dung beetles may face trade-offs that constrain a higher preference of nutrient-rich dung. Carnivore dung, for example, is more nutrient rich compared to herbivore dung (Fig. 5.1 – 5.3; (Whipple and Hoback 2012b)), but could contain pathogenic bacteria, which are perceived by the dung beetles via olfactory cues (Mansourian et al. 2016). The C/N ratio is frequently used as an index for quality descriptions of organic substrates including dung (Holter and Scholtz 2007). In general C/N ratios increased over ten-fold from carnivore dung (lowest value for mink, 5.0) to herbivore dung (highest value for donkey, 59.2); omnivore dung has intermediate levels (Fig. 5.1, Table 5.1). Corresponding to a higher nitrogen (N) content (i.e. the reverse of the C/N ratio), the amount of amino acids and thus a higher nutritional value (Elser et al. 2000) increased from carnivore to herbivore dung. Still, all dung types contained nearly all amino acids that are essential for insects (except for methionine in mink, otter and raccoon dung) and thus for dung beetles (Nation 2002), which finally results in differences for the amino acid composition solely on a feeding guild level (Fig. 5.3). Amino acids share key roles in insect development, such as the emergence from the pupal skin, and are precursors of pigments or for growth in larvae and adults (Gilmour 1961). Therefore our results highlight that dung is able to supply the beetles' need for amino acids in general - likewise for further synthesis and for direct use. Like N and amino acids, the amount of NLFAs increased over ten-fold from herbivores (goat and rabbit, 8 µg/mg) via omnivores to carnivores (lynx, 105 µg/mg). Additionally, dung types were characterized by feeding guild specific fatty acids such as unsaturated fatty acids (e.g. oleic and linoleic acid) for carnivores and omnivores, while herbivores showed higher amounts of saturated fatty acids (Table 5.3). Interestingly, there was no analytical indication for α-linolenic acid (C18:3, Δ9,12,15) in NLFAs and only in

trace amounts for FFAs. α-linolenic acid is thought to be one of the essential fatty acids for insects (Prosser 1991) and hence, needs to be consumed or supplemented by symbiotic bacteria. Although we could detect FFAs from C6:0 (caprylic acid) to C28:0 (montanic acid) we only found C16:0/C18:0 in notable amounts. These amounts were the same among all feeding guilds and also the overall lipids [µg/mg] showed a similar pattern to NLFA amounts. However, the FFAs/total lipid ratio (Fig. 5.2c) indicated more free fatty acids in herbivores than in carnivores, which hence may indicate different fat-digestive performance, but more importantly also foster different palatability among feeding guilds. This also further supports the view of Jones (2017), that dung is not waste, but a valuable resource for coprophagous beetles, which use beside other nutrients especially the present fatty acids during growth and larval emergence (Arrese and Soulages 2010, Verdu et al. 2010, Gilbert and O'Connor 2012). Water contents of different dung types were similar throughout the feeding guilds (except for gerbil dung that contained only 7% water) (Fig. 5.1, Table 5.1). Still, water content plays an important role, as adult dung beetles mainly use the liquid phase and its nutrients/particles to feed on (Holter 2016), it affects the occurrence of species (Gittings and Giller 1998) and the handling for brood balls (Hanski 1987). Insects, unlike other animals, lack the ability to synthesize sterols, and they must obtain such compounds via food or bacterial symbiont (Nation 2002). Sterols have several key functions, since they severe as components of the cell-membrane (especially cholesterol), as regulators of developmental genes and as precursors of different hormones (Behmer and Nes 2003). Our analysis confirmed sterols and cholesterol in all dung types, yet some amounts were extremely low, especially in herbivores. Moreover, carnivore dung seems to be a valuable resource regarding cholesterol (Fig. 5.2d-e), which normally (i.e. in herbivores) is metabolized from certain phytosterol (Behmer and Elias 1999a, Behmer and Elias 1999b, 2000, Jing et al. 2013). Thus, dung beetles may either obtain cholesterol directly from dung (especially carnivore dung) or synthesize it from sterols in herbivore/omnivore dung. The amount of sterols in dung may, however, be too low to fully supplement dung beetles, thus consumption of other food (e.g. plant material, (Rembialkowska 1982)) or bacterial symbiosis might help to acquire all mandatory sterols.

Conclusion

All nutrient parameters, C/N ratio, amino acids, fatty acids, cholesterol/sterol ratio and the composition of amino acids, showed substantial variation across dung types and feeding guilds. Although dung represents an already-digested, but still valuable resource it grants sufficient amounts of (essential) nutrients for insects. Hence, symbiotic bacteria may not be

mandatorily needed for nutritional upgrading. Regarding C/N, protein (= amino acid content) and fatty acids (= NLFAs and free fatty acids), dung showed similar values to resources available for other terrestrial beetles, such as litter, fruits, fungi and carcasses (see supplementary information; Table S5.1). We did not confirm that nutritional composition drives the beetles' food selection, however suggesting that the beetles' attraction to specific blends of volatiles may be uncoupled from nutrient values, and hence not serving as nutritional cues.

Ethics statement

Field work and animal collection permits were issued by the responsible state environmental offices of Baden-Württemberg, Thüringen, and Brandenburg (according to § 72 BbgNatSchG).

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5.6 Supplementary Information to Chapter 5

Table S5.1: Food plan of the animals whose dung was used in this study

species	origin	diet	feeding guild
lynx	wildlife park 'Alte Fasanerie'	meat of cattle, chicken, rabbits, pigeon and deer	carnivore
mink	zoo 'Opel-Zoo'	meat of chicken, mice, rats, pigeon and freshwater fish	carnivore
otter	zoo Vivarium	freshwater fish, rats, mice	carnivore
raven	zoo Vivarium	rats, mice	carnivore
snowy owl	zoo Vivarium	rats, mice	carnivore
wildcat	zoo 'Opel-Zoo'	meat of chicken, mice, rats, pigeon and freshwater fish	carnivore
wolf	wildlife park 'Alte Fasanerie'	meat of cattle, chicken, rabbits, pigeon and deer	carnivore
brown bear	Fasanerie Wiesbaden	vegetables, fruits, occasionally meat and fish	omnivore
chicken	private stock	wheat, maize, seeds, salad, grass, vegetables, mealworms, eggshells and chalk (grit)	omnivore
fox	wildlife park 'Alte Fasanerie'	60 % meat (chicken, mice, rats, cattle), fruits, vegetables	omnivore
gerbil	private stock	mealworms, carrots, seed mixture (oat, linseed, lucerne)	omnivore
raccoon	zoo 'Opel-Zoo'	fruits and vegetables, eggs; occasionally meat	omnivore
wild boar	wildlife park 'Alte Fasanerie'	pig food (raiffeisen), bread, maize, fruit, vegetables, lucerne pellets, meat of cattle & deer and red deer	omnivore
cow	Oberfeld farm (organic)	grazing on pasture, hay	herbivore
deer	wildlife park 'Alte Fasanerie'	grass, hay, maize, fodder beet, lucerne pellets, apples, carrots	herbivore
donkey	zoo Vivarium	fresh and dried fruits, hay silage, fresh hay, grazing on pasture, maize, barley, oat	herbivore
elephant	zoo 'Opel-Zoo'	grass, hay, oat, wheat bran, apples, carrots, branches; occasionally lucerne hay, mineral briquet	herbivore
elk	zoo 'Opel-Zoo'	lucerne hay, moose-pellets (mazuri), leaves and bark	herbivore
goat	zoo Vivarium	hay silage, fresh hay, grazing on pasture, maize	herbivore
horse	Oberfeld farm (organic)	grazing on pasture	herbivore
rabbit	private stock	carrot, salad, grass, herbs	herbivore
sheep	Oberfeld farm (organic)	grazing on pasture	herbivore
wisent	wildlife park 'Alte Fasanerie'	grazing on pasture, hay	herbivore

Supplementary Methods S1: Detailed methods of dung sampling and chemical analyses

Dung used in this study

We used 23 different dung types of carnivorous, omnivorous and herbivorous species, namely: brown bear (*Ursus arctos* L., 1758), chicken (*Gallus gallus domesticus* L., 1758), cow (*Bos taurus* L., 1758), donkey (*Equus africanus asinus* L., 1758), elephant (*Loxodonta africana* Blumenbach, 1797), elk (*Cervus canadensis* Erxleben, 1777), fox (*Vulpes vulpes* L., 1758), gerbil (*Meriones unguiculatus* Milne-Edwards, 1867), goat (*Capra aegagrus hircus* L., 1758), horse (*Equus caballus* L., 1758), lynx (*Lynx lynx* L., 1758), mink (*Mustela lutreola* L., 1761), otter (*Aonyx cinerea* Illiger, 1815), rabbit (*Oryctolagus cuniculus domestica* L., 1758), raccoon (*Procyon lotor* L., 1758), raven (*Corvus corax* L., 1758), red deer (*Cervus elaphus* L., 1758), sheep (*Ovis aries* L., 1758), snowy owl (*Bubo scandiacus* L., 1758), wild boar (*Sus scrofa* L., 1758), wild cat (*Felis silvestris silvestris* Schreber, 1777), wisent (*Bison bonasus* L., 1758) and wolf (*Canis lupus* L., 1758). Game species´ and domestic animals´ dung was collected in wildlife parks, zoos or came from private stocks. Since the captive animals' diet may differ from those of wild animals, we collected our dung samples only from organic farms and wildlife parks / zoos where the animals are kept on most natural diets without food additives and concentrated feeding stuff. The detailed food plan can be found in supplementary table S5.2.

Dung sampling in the Biodiversity Exploratories

For dung beetle field samplings we used pitfall traps equipped with the dung baits of 12 different representative subsamples (i.e. dung available in sufficient amounts) collected form mammal species, namely: wolf, lynx, fox, brown bear, wild boar, cow, horse, sheep, deer, elephant, elk and wisent (2 carnivores, 3 omnivores and 7 herbivores). The traps were set up randomized on a transect, in a total of 54 experimental sites (27 in forests, 27 in grasslands) in three regions of Germany. The regions are: (1) Biosphere Reserve Schorfheide-Chorin (in North-East Germany, ~13.000 km2, 3 – 140 m a.s.l., 13°23'27''–14°08'53'' E / 111 52°47'25''–53°13'26'' N), (2) Hainich National Park and surroundings (in Central Germany, ~13.000 km2, 285 – 550 m 112 a.s.l., 10°10'24''–10°46'45'' E / 50°56'1 4''–51°22'43'' N) and (3) Biosphere Reserve Schwäbische Alb (in South-West Germany, ~422 km2, 460 – 860 m a.s.l., 09°10'49''–09°35'54'' E / 114 48°20'28''– 48°32'02'' N).

Lipid analyses

Total neutral lipids (hereafter, neutral lipid fatty acids = NLFAs) were extracted from the fresh dung samples (40 - 50 mg of fresh weight) using 1 ml of a chloroform:methanol-mixture, 2:1 (V/V) over a period of 24 h. Afterwards extracts were purified and separated using SiOH-columns (Chromabond® SiOH, Macherey-Nagel GmbH & Co. KG, Düren, Germany) which were washed and conditioned with 6 ml hexane. Afterwards samples were applied on the column and elution of neutral lipids was accomplished with 4 ml of chloroform. Afterwards the chloroform fraction was evaporated to dryness under nitrogen gas flow and residuals were redissolved in dichloromethane:methanol, 2:1 (V/V) using different amounts of solvent depending on the dung-type (200 µl for herbivores, 500 µl for omnivores and 750 µl for carnivores) to adjust the concentration. 20 µl were transferred to chromatographic glass vials with a conical inlet (150 µl), 20 µl nonadecanoic acid (220 ng/µl) was additionally added as internal standard and the mixture was evaporated to dryness again and subsequently derivatized to fatty acid methyl esters (FAMEs) with TMSH (trimethylsulfonium hydroxide; 0.25 M in MeOH from Fluka, Sigma-Aldrich, St. Louis, USA) reagent according to the supplier's information. Free fatty acids (= FFAs) were extracted as described for NLFAs (see above), however we had to exclude raven and snowy owl from this analysis. Afterwards extracts were purified and separated according using SiOH-columns (Chromabond® SiOH, Macherey-Nagel GmbH & Co. KG, Düren, Germany) which were washed and conditioned with 6 ml hexane. Afterwards samples were applied on the column and triglyceride/sterol- and diglyceride-fractions where eluted with 4 ml isooctane:ethyl acetate (10:1, V/V) and 4 ml isooctane:ethyl acetate (3:1, V/V), respectively and discarded afterwards. Free fatty acids were finally eluted with 4 ml of a solvent mixture containing isooctance:ethyl acetate:acetic acid (75:25:2; V/V/V). Subsequently the final solvent fraction was evaporated to dryness under nitrogen gas flow and residuals were redissolved in dichloromethane:methanol, 2:1 (V/V) and further processed as described above for NLFAs.

Amino acids

For analysis of the amino acids (free amino acids and protein-bounded), 5 mg (± 0.1 mg) dried dung was diluted in 200 µL of hydrochloric acid (6 mol/l) and boiled for four hours at 100°C, and cooled to room temperature afterwards. Note that this acidic chemical extraction decays asparagine, glutamine and tryptophan. Afterwards residuals were cooled to room temperature, centrifuged (10 min at 14,800 rpm) and the supernatants were transferred

into fresh tubes and were evaporated to dryness at 100°C, before the samples were re-dissolved in 200 µL of deionized water and evaporated repeatedly. Samples subsequently were re-dissolved again in 200 µL of deionized water and finally, the amino acids were measured with an ion exchange chromatograph with ninhydrin post-column derivatization (Biochrom 20+, Amino Acid Analyzer, Cambridge, UK). A standard amino acid mixture (Laborservice Onken GmbH, Gründau, Germany) was used as external standard. The amount of total amino acids [µg] was standardized using the dry weight [mg] of the initial sample.

Table S5.2: Variation of nutrient concentrations within each dung type (sampled at different times and/or locations) expressed as coefficient of variation (CV = sd/mean). Mean values across all dung types shown for within-dung type variability (CV_{within}) and across-dung type variability (CV_{across}). In parentheses, range of CVs (min – max) shown across different dung types (^{D}) or different substances (^{S}). Nutrient variation across dung types was 3.4 to 7.6-fold higher across dung types than within. The mean number of replicates per dung type (*n*) is provided and its range in parentheses; CVs were calculated for all dung types where $n > 1$.

	n	CV_{within}	CV_{across}
Total amino acids	4 (2 – 10)	0.26 (0.03 - 0.67)^{D}	1.11
Single amino acids (14 substances)	4 (2 – 10)	0.30 (0.23 - 0.41)^{S}	1.15 (0.76 - 1.44)^{D}
Fatty acids and sterols (24 substances)	1.8 (1 – 4)	0.49 (0.13 - 0.92)^{S}	1.66 (0.60 - 3.19)^{D}
C/N ratio	5.1 (3 – 15)	0.09 (0 - 0.37)^{D}	0.71
Water content	2 (1 – 4)	0.10 (0 - 0.72)^{D}	0.34

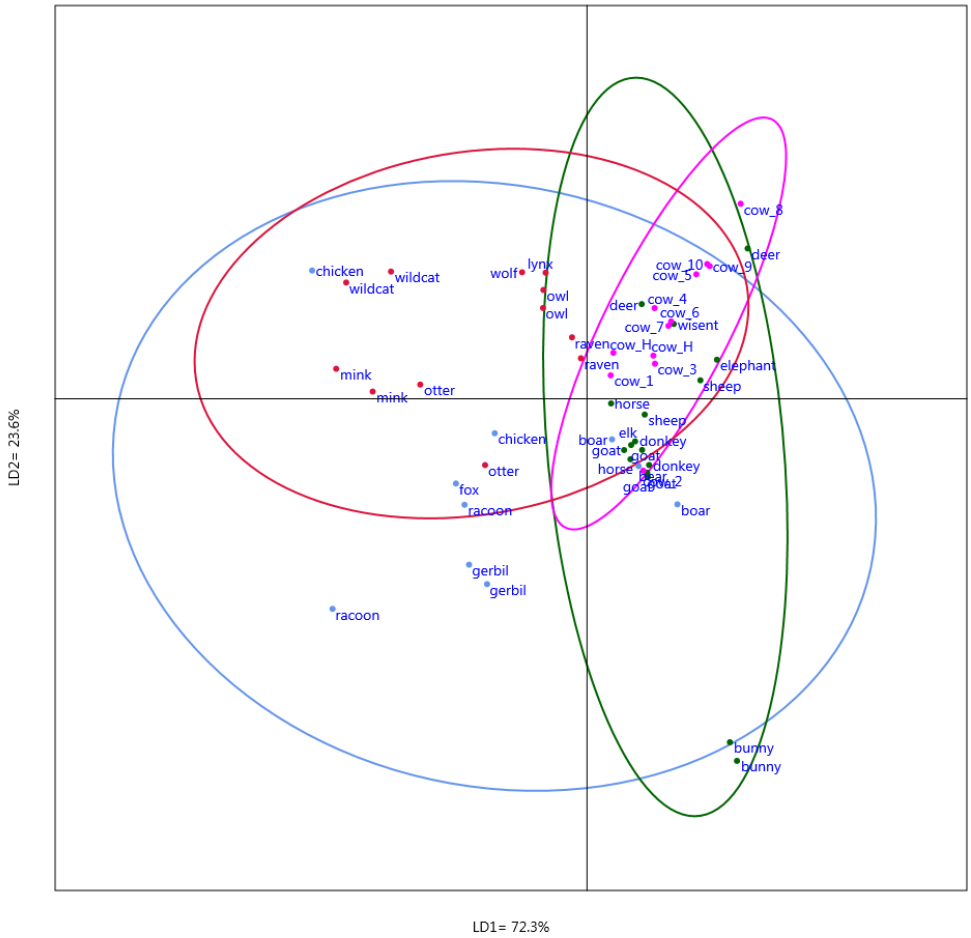


Figure S5.1: Discriminant analysis of principal components (DACP) for and neutral lipid fatty acids, including all batch replicates. Groups are clustered in red for carnivore, in blue for omnivore and in green for herbivore dung. An additional cluster in pink shows cow dung from 11 different farms.

List S5.1: Linear regression formulas for dung dry weight calculation

Note: Always three point calibration, R^2=0.98-0.99

1 Cow: dw = 0.1764 x fw – 0.6322

2 Fox: dw = 0.4598 x fw + 2.4886

3 Wild boar: 0.6617fw-0.3902

4 Deer: dw= 0.4057fw-1.2012

5 Horse: dw=0.2549fw-0.7973

6 Sheep: dw=0.2881fw+0.5172

7 Lynx dw=0.3845fw+0.0083

8 Elephant dw=0.2608fw-1.3293

9 Elk dw=0.2897fw-0.1194

10 Wisent dw=0.2132fw-2.5741

11 Wolf dw=0.6352fw-0.8341

12 Bear dw=0.2397fw—0.98

13 Raven dw=(0.733fw+6.8379)*(100/27) # with correction for non-organic material

14 Otter dw=0.5547fw-16.707

15 Owl dw=0.6098fw+13.627*(100/16) # with correction for non-organic material

16 Donkey dw=0.1194fw+6.1563

17 Goat dw=0.5295fw+1.3208

18 Gerbil dw=0.879fw+4.1493

19 Bunny dw=0.2872x+1.9051

20 Chicken dw=0.5277fw-5.7143

21 Mink dw=0.4218fw+24.672

22 Racoon dw=0.461fw-4.5469

23 Wildcat dw=0.4484fw+6.4164

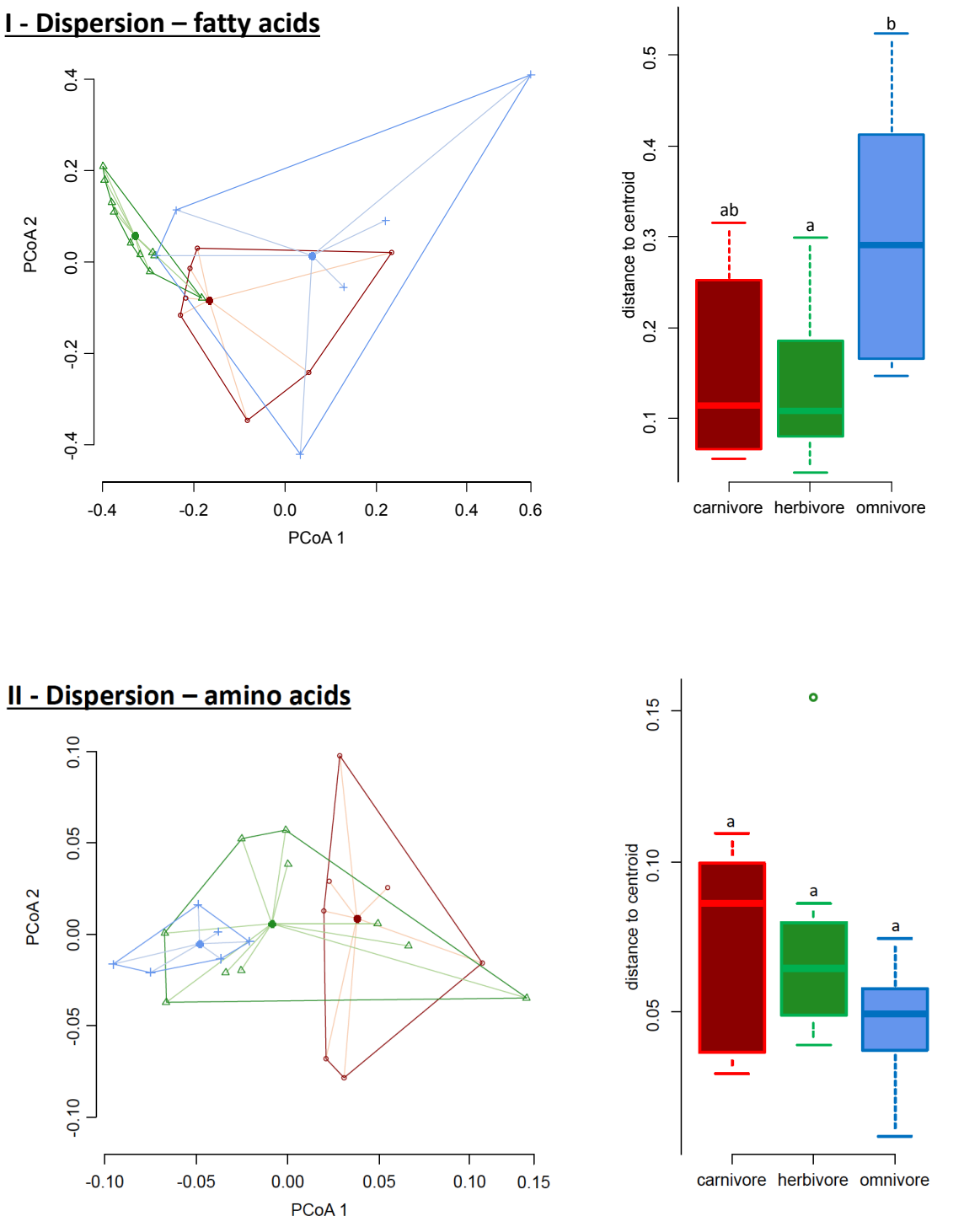
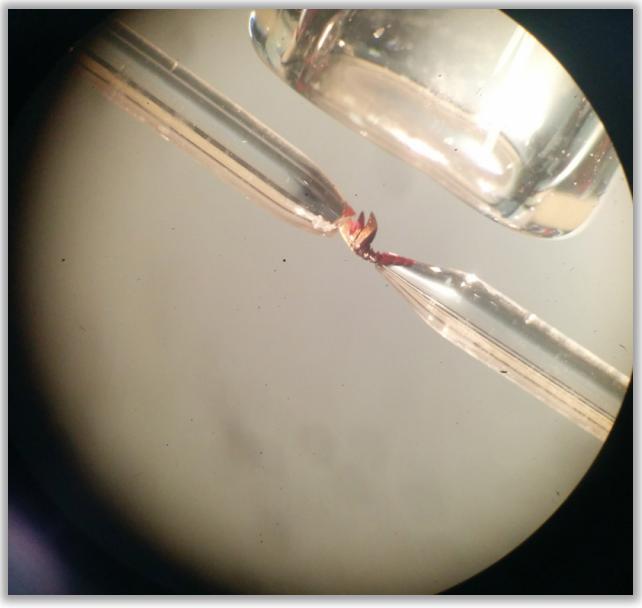


Figure S5.2: Dispersion for fatty acids (I) and amino acids (II) in carnivore, omnivore and herbivore dung samples.

Table S5.3: C/N ratios, protein and fat content of other food sources potentially used by dung beetles.

Substrate	**C/N ratio**	**Protein [µg/mg]**	**Fat [µg/mg]**	**Reference**
leaf litter	40	13	5	(Bridson 1985, Aerts 1997, Rawlins et al. 2006)
conifer litter	62	-	8	(Bridson 1985, Aerts 1997)
apple fruit	31	3	4	(Md Khudzari et al. 2016), Council directive: 90/496/EEC (1990)
fungi	8	109	15	(Longvah and Deosthale 1998, Mouginot et al. 2014), Sadler (2003)
pig cadaver	8	180	150	(Enser et al. 1996, Carter et al. 2007)
bone meal	4	480	80	(Gotaas 1956, Hendriks et al. 2002)
dung beetle body	4	544	136	(Raksakantong et al. 2010), Blüthgen unpublished

Chapter 6



6 In search for cues: dung beetle attraction and the significance of volatile composition in dung

Kevin Frank, Adrian Brückner, Nico Blüthgen and Thomas Schmitt

6.1 Abstract

Volatile organic compounds (VOCs) provide multiple informations for animals such as food location, type, and condition of resources. Hence, especially for resources with a patchy distribution, scents are often crucial for localization and discrimination by the consumer. Dung beetles (Scarabaeoidea) rely on such scented resources. Despite the beetles' (mostly) generalistic choice across dung types, several studies showed that the beetles prefer some dung types over others. Yet, the role of VOCs during dung localization and differentiation remains unclear. In this study, we used six single chemical components (indole, skatole, phenol, butyric acid, 2-butanone and p-cresol), two blends of those and six different dung types for a detailed analysis of dung beetle attraction. We found that dung baits and baits with synthetic compounds were able to attract similar communities, however, there are large differences in the number of attracted species and their abundance. Still, we found (almost) no specialization of beetle species towards certain VOCs. The analysis of dung scent profiles revealed both, unique patterns in composition and ubiquitous components, such as p-cresol. Finally, we found a large blend of VOCs, including six single components, being as attractive as three of the most attractive dung types in the field. Hence, our findings highlight the usage of key VOCs, but moreover that dung beetles use a mixture of components for resource localization.

6.2 Introduction

Detritivorous diets are either abundant resources with low in energetic value (litter) or high in energy but sporadic and ephemeral (animal droppings or carcasses). Consequently, the majority of dung-feeding (coprophagous) insects are most likely generalistic polyphages

(Hanski and Cambefort 1991). In contrast, to this suggested generalistic selection and use of animal droppings, dung beetles (Coleoptera: Scarabaeoidea) show preferences in the choice and utilization of different dung types (Hanski and Cambefort 1991, Martín-Piera and Lobo 1996, Galante and Cartagena 1999, Whipple and Hoback 2012a, Frank et al. 2017b). This phenomenon of "choosy generalism" is quite common among other detritivores and decomposers, like collembolans or mites (Klironomos et al. 1992, Schneider and Maraun 2005). Yet such a strategy could only evolve and persist if dung types vary in cue quality or intensity (= quantity), allowing dung beetles to respond to or distinguish among different types of dung resources.

Volatile organic compounds (VOCs) provide multifaceted information for organisms, for instance location, type and condition of resources (Schmitt et al. 2004, Verdu et al. 2015, Jones 2017). Hence, especially for ephemeral and scarce resources, odors composed of specific VOCs are mandatory tools for localization and decision-making (Vet and Dicke 1992). Accordingly, dung beetles rely on scenting resources, as they perform cruising flights to find dung and finally perceive VOCs *via* olfactory sensillae (Inouchi et al. 1988, Gill 1991). Dung scents are composed of a vast number of different VOCs, yet some compounds appear to be quite common in all dung types, potentially providing cues for generalistic foraging behavior in dung beetles (Dormont et al. 2007, 2010, Stavert et al. 2014). However, some substances appear to be specific for a particular dung type (Dormont et al. 2007, 2010), providing cues for a selective generalist behavior of dung beetles. Additionally, olfactometer bioassays with two different dung types (Dormont et al. 2004, 2007) support the hypothesis of a "choosy generalism" for dung beetles and showed that respective dung scents allow identification and choice of specific dung types. Identified dung-VOCs (as single or mixed chemical components) can be used to attract dung beetles in the field, however dung baits appear to attract a broad range different dung beetle species in high abundances (Wurmitzer et al. 2017). Thus the complexity of dung scents, but also certain key components might be important for dung attractiveness (Wurmitzer et al. 2017). Given that there are still only scarce information whether dung beetles use individual scents or multi-compound blends to locate and discriminate among preferred dung resources (see also Stavert et al. 2014, Wurmitzer et al. 2017), it is not yet possible to depict a VOC-based scenario that would conclusively explain the choosy generalistic foraging in dung beetles.

In this study we use single and mixed chemical components in a comparative large-scale field approach together with different types of dung to test the attractiveness of certain

VOCs as well as complex VOC profiles for dung beetle communities in Central Europe. In addition to single chemical components found in dung scents, we also used specific mixtures as well as dung of six different mammal species (cow, sheep horse, deer, wild boar and fox) to crosslink synthetic VOC blends with natural dung bouquets. In a previous study (Frank et al. 2017b) we found that all used dung types are generally attractive to a broad spectrum of dung beetle species, yet again a variable number of individuals was attracted. Since dung attractiveness was unrelated to the dungs' nutrient quality, i.e. the amount and composition of amino-, fatty acids, as well as sterols (Frank et al. 2017a), we used a volatile-driven approach to investigate patterns in the dung beetles' resource preference.

We therefore tested (1) if dung beetle communities are attracted by specific VOC-baits (i.e. show a specialization), compared to dung. We (2) analyzed dung specific volatiles and tested for similarities in dung scent profiles compared to bait VOCs. We tested (3) whether observed patterns in dung attractivity are explainable by the dungs' VOC-compositions. Finally, since dung odor contains a large number of different VOCs, we tested (4) the attractivity of multiple volatiles in comparison to single compounds and natural dung samples.

6.3 **Material and Methods**

Experimental design

To assess dung beetle abundance and preference towards different bait types, we used dung baits and scent baited pitfall traps (hereafter: chem baits), including single chemical components and mixtures. Six different dung types consisting of three livestock and three game species were used, namely: cow (*Bos taurus* L., 1758), horse (*Equus caballus* L., 1758), sheep (*Ovis aries* L., 1758), red deer (*Cervus elaphus* L., 1758), wild boar (*Sus scrofa* L., 1758) and fox (*Vulpes vulpes* L., 1758). For the chem baits we used six single components, namely: 2-butanone, butyric acid, phenol, indole, skatole and p-cresol. All components are known to occur in dung samples and (partly) trigger reactions in the beetles' antennal olfactory cells (Inouchi et al. 1988, Dormont et al. 2007, Dormont et al. 2010, Stavert et al. 2014). Additionally, we created two chem baits by blending four components (small blend: 2-butanone, butyric acid, indole, skatole) and all six components (large blend), to test for enhanced attractivity with a rising number of volatiles. Livestock dung was collected at the farm 'Oberfeld' in Darmstadt (cow and horse) and at a sheep farm in Darmstadt (sheep).

Game species dung has been collected in the wildlife park ‘Alte Fasanerie’ in Hanau (fox, wild boar and red deer) and at the zoo ‘Opel-Zoo’ in Kronberg (additional fox). To prevent compromising baits due to medication (Lumaret et al. 2012, Verdu et al. 2015), we ensured no medical treatment for several weeks before dung sampling (interviews with farmers and animal keepers). For each bait dung was filled in a tea bag (approx. 35 g, Rubin, Burgwedel, Germany) and transferred in a freezer bag. Afterwards the freezer bags where hermetically sealed, labelled and stored at -20 °C until use, to prevent microbial decomposition or moulding. For the chem baits we used 0.5 ml for liquid components (2-butanone, butyric acid) and approx. 0.5 ml of pure powdery components (indole, skatole, phenol, p-cresol).

Study Site

This study was conducted within the framework of the Biodiversity Exploratories project, comprising three regions with representative forest and grassland sites in north-east, central and south-west Germany. The three regions are: (1) Biosphere Reserve Schorfheide-Chorin (in North-East Germany, ~13.000 km^2, 3 – 140 m a.s.l., 13°23’27’’–14°08’53’’ E / 52°47’25’’–53°13’26’’ N), (2) Hainich National Park and its surroundings (in Central Germany, ~13.000 km^2, 285 – 550 m a.s.l., 10°10’24’’–10°46’45’’ E / 50°56’14’’–51°22’43’’ N) and (3) Biosphere Reserve Schwäbische Alb (in South-West Germany, ~422 km^2, 460 – 860 m a.s.l., 09°10’49’’–09°35’54’’ E / 48°20’28’’– 48°32’02’’ N). Within each region, 100 square-shaped experimental sites (hereafter: sites) were chosen at random, 50 sites in forests (each 100 x 100 m) and 50 in grasslands (50 x 50 m). In total, the study sites cover approx. 10% of Germany’s agricultural land, pastures and forests. For more details of the Biodiversity Exploratories see Fischer et al. (2010). To account for the whole dung beetle community and depict a representative selection of their habitats we sampled a subset of 54 sites (9 forests and 9 grasslands per region). Our sampling took place in April 2015 (06.04. – 24.04.).

On each site we placed 14 pitfall traps (six dung types and eight chem baits). Within each setup the baits were placed randomly by a distance of 10 m. The traps were made of plastic cups (vol.: 500 ml) with inserted dome lids as funnels and tea bags filled with either dung or tissue paper covered with the corresponding chem bait, attached to a skewer by an elastic strap. We placed the traps at ground level and took care, that there was no barrier for walking beetles. Each bait was inaccessible approx. 10 cm above the center of the trap. In each site experimental setups remained for 48 h. Afterwards trapped beetles were collected,

labelled (date, site-ID, bait type) and stored in a freezer at -20 °C. In the lab dung beetles were identified to species level based on literature (Freude et al. 1969, Bunalski 1999, Rössner 2012) and with the help of taxonomic experts (see Acknowledgements).

Dung volatiles

For the extraction of dung specific volatiles we used approx. 30 g of fresh dung for each dung type. The dung sample was placed on a petri dish and covered with a reversed funnel (both made of glass). The whole setup was covered and sealed in a polyester tube (PET, Toppits; Minden, Germany). To ensure a steady airflow and avoid contamination we used a charcoal filter embedded in the tube (Charcoal activated granular; AppliChem, Darmstadt, Germany). Dung volatiles were trapped with a ChromatoProbe-Filter system containing a mixture of Tenax and Carbotrap (1.5 mg of each adsorbent; Supelco, Munich, Germany) (Dötterl *et al.* 2005), which was attached at the pointed end of the funnel. A continuous airflow was led for 4 hours through the filter system [using a vacuum pump (Vacuubrand MZ 2C, Wertheim, Germany)] with approx. 100 ml min^{-1}. The adsorbed volatiles were eluted with 3x50 µl pentane/acetone (9:1) (Merck KGaA, Darmstadt, Germany, > 99.5 % purity). The solvent was evaporated to approx. 20 µl under a gentle stream of nitrogen for further analysis. Two dung sample replicates for each dung were analyzed with a QP2010 Ultra GC/MS (Shimadzu, Duisburg, Germany). The gas chromatograph was equipped with a ZB-5MS fused silica capillary column (30 m x 0.25 mm ID, df= 0.25 µm) from Phenomenex (Aschaffenburg, Germany). 3 µl sample aliquots were injected by using an AOC-20i autosampler-system from Shimadzu, Duisburg, Germany into a programmable temperature vaporizing-split/splitless-injector (Optic 4, ATAS GL, Eindhoven, Netherlands), which operated in splitless-mode. The injection-temperature was 40°C (5 sec hold) and was raised to 230 °C with a heating rate of 20°C/sec and then an isothermal hold for 30 minutes. Hydrogen was used as carrier-gas with a constant flow rate of 3.07 ml/min. The temperature of the GC oven was raised from initial 40°C for 1 min, to 230°C with a heating-rate of 7.5°C/min and then an isothermal hold at 230°C for 5 min. Electron ionization mass spectra were recorded at 70 eV from m/z 40 to 350. The ion source of the mass spectrometer and the transfer line were kept at 230°C and 300°C, respectively. Identification of dung specific volatiles was carried out using the mass spectral data bases NIST 11, Wiley 9, MassFinder 3, FFNSC 2, and Adams. Whenever possible, components were verified using retention indices and mass spectra of authentic standards, by comparison with published retention indices. Contaminates were excluded based on the negative control collected from laboratory air.

Data analysis

Data analyses were conducted with the statistical software R 3.3.2 (R Core Team 2016). For sampling completeness we used an abundance-based coverage estimator (ACE) according to Chao and Lee (1992), while for community analyses (species richness, Shannon diversity) and to test for correlations between sampled communities and scent composition of each dung type (Mantel test) we used the R package 'vegan' (Oksanen et al. 2007). The variation in species-specific preferences for certain dung types or scents across dung beetles was assessed by the standardized complementary specialization index (H_2`) (Blüthgen et al. 2006, Blüthgen et al. 2007), see also (Wurmitzer et al. 2017). Additionally, we used the overall sum of sampled beetles with dung baits and the sum of sampled beetles for each specific chem bait to test for a bait type driven specificity (dung versus chem bait). The corresponding H_2' provides the degree of specialization for the sampled dung beetle community between overall dung and single chem baits.

We used PAST 3 (Hammer et al. 2001) to test for differences in bait attractivity (Wilcoxon paired test, Bonferroni corrected) and differences in community composition between dung- and chem baits (PERMANOVA on Bray-Curtis similarities (BCS)). The mean compositional data (in %) was subjected to cluster-analysis using UPGMA on BCS. We are aware of habitat preferences for dung beetles (Frank et al. 2017b), thus we pooled the whole data in our analyses (for all regions and habitats) to account for a complete dung beetle community and avoid compromising effects, driven by the beetles' occurrence in the field. Trends for community comparison (H_2') remain significant after exclusion of *A. prodromus*, the most dominant species.

6.4 **Results**

In total, we collected 5727 individuals from 21 species of dung beetles on 54 experimental sites. 3714 individuals (21 species) were collected with dung baits, while 2013 individuals (10 species) were collected with chem baits (supplementary table S1 & S2). The abundance-based estimates of the species richness for dung baits (ACE) showed a sampling completeness of ≥ 86 %, whereas chem baits showed a sampling completeness of ≥ 75 % (large blend, small blend, butyric acid, p-cresol), except for skatole with 67%. 2-butanone; indole and phenol could not be estimated due to too low numbers of sampled individuals.

Dung baits attracted significantly higher numbers of dung beetles than chem baits (Fig. 1, dung baits: 68.8 ± 9.4, chem baits: 37.3 ± 12.3; Wilcoxon two-sample paired test, z = 4.06; $P < 0.0001$). Whereas species richness (z = 5.7, $P < 0.0001$) and effective Shannon diversity (z = 4.2, $P < 0.0001$) were different, the dung beetle community composition showed no differences between dung baits and chem baits (PERMANOVA on BCS; permutations = 10.000, $_{pseudo}F = 1.44$, $P = 0.24$).

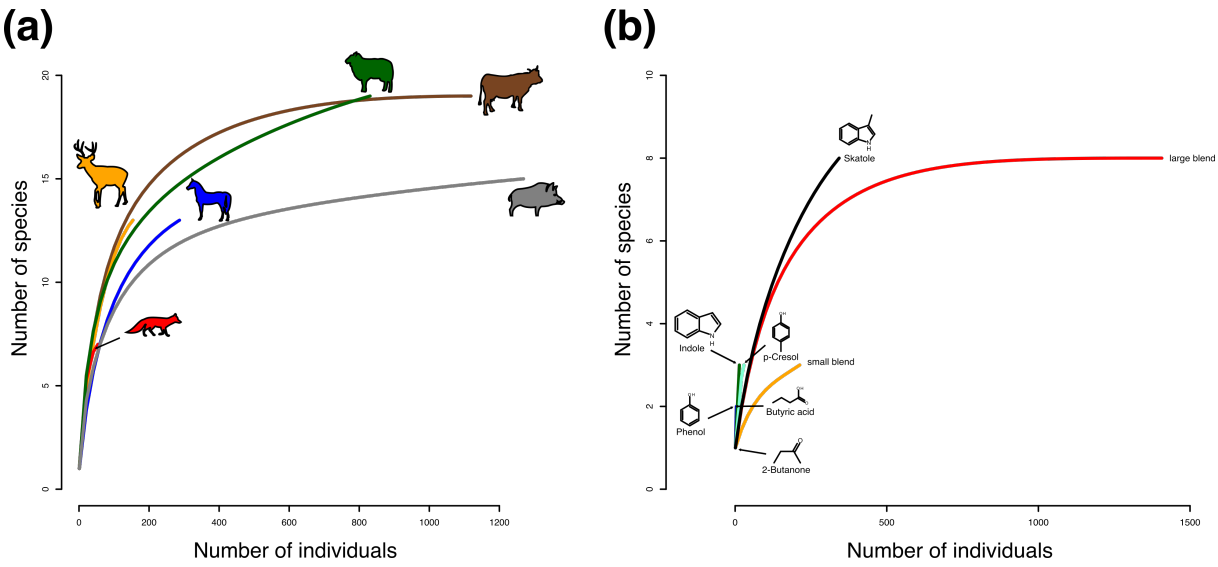


Figure 6.1: Rare faction curves show the number of collected species (including the number of individuals per species) for (a) dung baits (wild boar, cow, sheep, horse, deer, fox) and (b) chem baits (large blend, skatole, small blend, p-cresol, indole, phenol, butyric acid, 2-butanone).

Dung beetle species were very similar in their dung type or volatile preferences, resulting in networks that indicate a very low complementary specialization (dung network: $H_2' = 0.102$, chem network: $H_2' = 0.108$) (Fig. 2a & b). Yet comparing the sum of dung baits and each specific chem bait of the sampled dung beetle communities (H_2') showed altering differences. While for skatol, large blend and small blend baits the sampled species differed significantly from the dung-baited community (all $P < 0.0001$), the specialization of the beetles for the baits was low ($H_2' = 0.07$, $H_2' = 0.14$, $H_2' = 0.07$, respectively). Butyric acid showed significant differences ($P = 0.002$), but H2' was comparatively higher ($H_2' = 0.35$). All other compounds showed no significant differences and highly variable specialization (p-cresol: $H_2' = 0.04$, $P = 0.10$; phenol: $H_2' = 0.11$, $P = 0.28$; 2-butanone: $H_2' = 0.35$, $P = 0.002$; indole: $H_2' = 0.02$, $P = 0.66$).

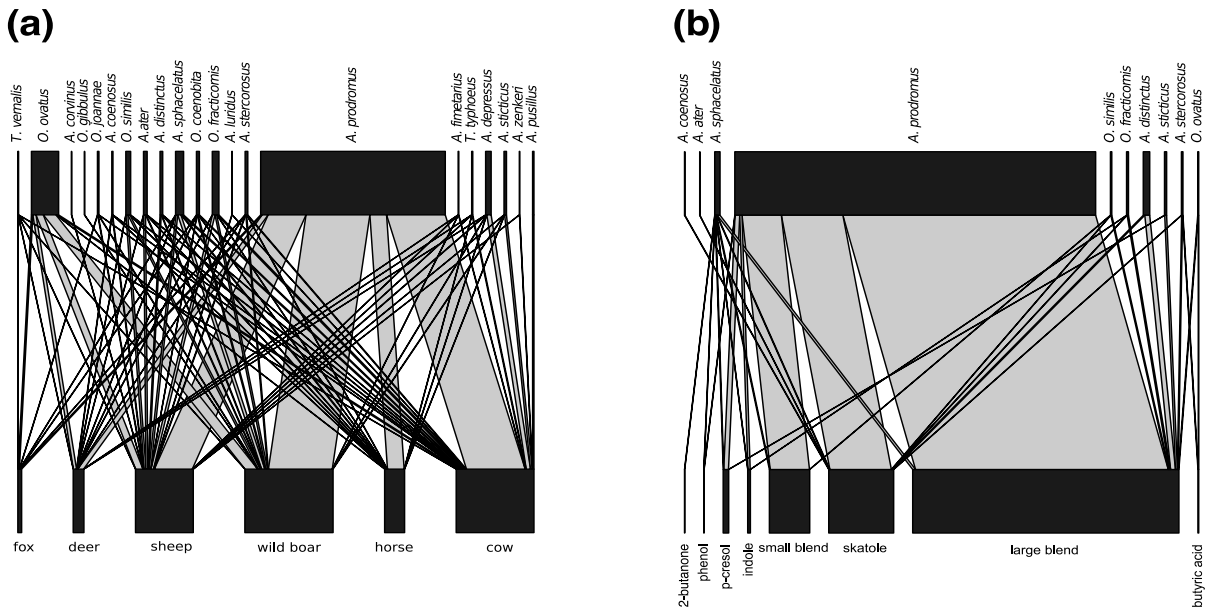


Figure 6.2: Species-resource interaction networks show links for each dung beetle species (top row) towards dung baits (a) and chem baits (b). Bar width and link strength represent the sum of all individuals sampled with the corresponding bait.

The similarity analysis of the scent composition showed two clusters (1st: horse, sheep, deer; 2nd: cow, fox, wild boar) (Fig. 3a, see supplementary table S3 for dung-related compound identification), which rely on the main VOCs of the dung scent (Fig. 3b & c). However, we found no correlation between the composition of the dung beetle community sampled with dung baits and the scent compositions of the dung types (Mantel test, permutations = 1000, $R = 0.41$, $P = 0.16$).

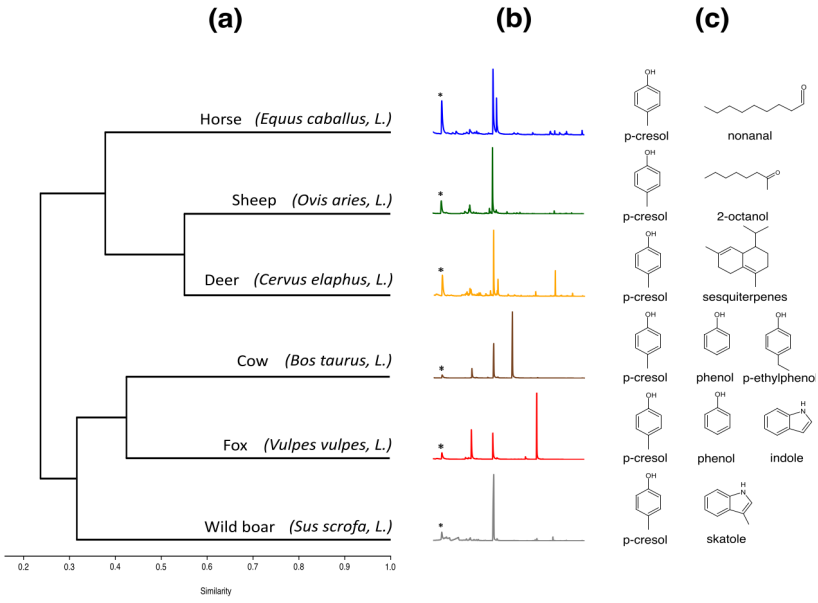


Figure 6.3: Similarity of volatile organic compound (VOC) profiles of dung (a) revealed two clusters (1st: horse, sheep, deer; 2nd: cow, fox, wild boar) displayed in a cluster dendrogram (using UPGMA on Bray-Curtis similarities). (b) presents gas-chromatographic traces of the dung scents (asterisks mark nonan, which was also found in air blanks), while (c) highlights chemical structures of the most abundant VOCs of the scent profiles.

For the attractivity of dung- and chem baits we found significant differences in sampled individuals among the baits (Friedman test; dung baits: $\chi^2 = 130.5$, df = 5, $P < 0.0001$; chem baits: $\chi^2 = 184.21$, df = 7, $P < 0.0001$) (Fig. 4). We found fox and deer dung being least attractive, while horse and sheep showed intermediate, cow and wild boar dung the highest attractivity. Chem bait attractivity showed a similar variation: 2-butanone, phenol, butyric acid and indole attracted (almost) no individuals, p-cresole, skatole and the small blend attracted an intermediate number while the large blend was the most attractive bait. Finally, the amount of collected beetles with the large blend did not differ significantly from the three most attractive dung baits - wild boar, cow and sheep (Fig. 4, Wilcoxon pairwise test, $z \geq 1.49$, $P \geq 0.14$).

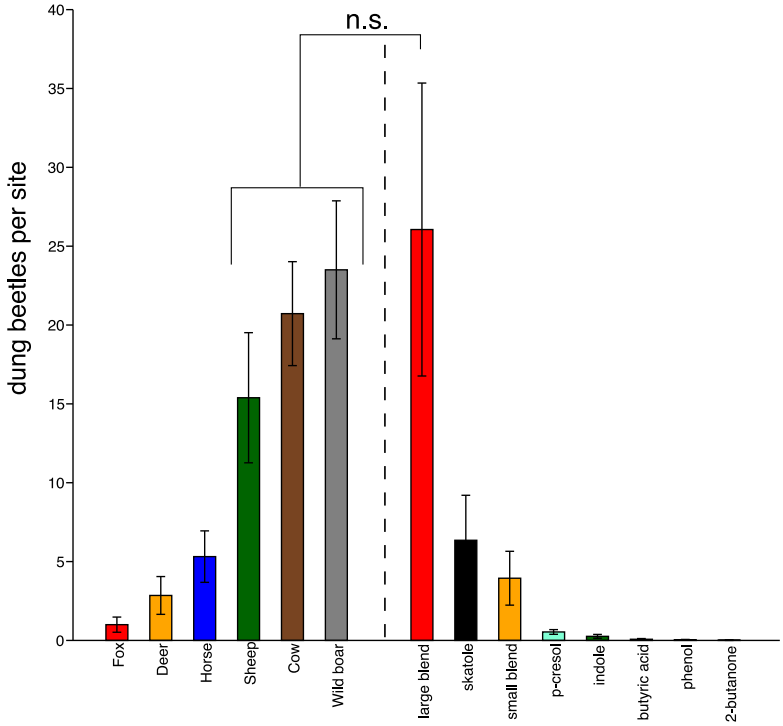


Figure 6.4: Mean number of collected individuals per site and bait type (left side: dung baits, right side: chem baits). The amount of collected individuals for the large blend (including all six bait-VOCs of the study) did not differ significantly from the three most attractive dung types (wild boar, cow and sheep). Error bars represent standard errors.

6.5 **Discussion**

Dung baits outnumbered the chem baits regarding the number of trapped species and abundance of individuals (Fig. 1a & b); also the abundance of dung beetles was different across all dung baits. Despite these differences, all dung types are used as a resource (Frank et al. 2017b), which again supports the hypothesis of choosy generalism in dung beetles (Dormont et al. 2004, Dormont et al. 2007). For the sampled beetle communities we found almost identical network complexity among all dung and chem baits, including a low degree of specialization towards the offered resources (dung and chem baits) (Fig. 2). This emphasizes the broad attractivity of the chosen dung types and scent. However, the effective

Shannon and species richness of the sampled communities remained significantly lower for chem baits compared to dung baits, mainly driven by much lower total abundance of beetles caught with the chem baits (see also Wurmitzer et al. 2017).

As the chosen chem baits were able to attract only a subset of dung beetle species sampled with dung baits, this indicates a "gap" for the volatiles used in dung beetle foraging. The dung beetle communities showed no specialization towards certain components or blends with a few exceptions (see H2' for single baits (results) & (Fig. 2). For instance, 2-butanone, was claimed to be one of the most volatile and attracting VOCs (Inouchi et al. 1988), yet we did not find it in any dung (and the compounds appears to only occur in trace amounts in nature, Neier and Strehlke 2002) and was one of the least attractive components in our study. Butyric acid on the other hand, showed a high specialization of attracted beetle species (see also Wurmitzer et al. 2017). However, the sampled number of beetles was very low, which makes it difficult to generalize these results. The large blend and skatole attracted more than twice of the number of baited species. Taking into account the number of individuals attracted, this suggests that certain VOCs trigger the attraction of dung beetles, but a way more complex bouquet is needed for an optimized, overall attraction.

The volatile composition of different dung types, however, did not explain the composition of the sampled dung beetle community, suggesting that different species use different blends to locate the resources. This suggestion is also supported by the number of species and individuals, sampled with our selection of chem baits. Regarding the chem baits, preferences of dung beetles revealed little to no attraction for most of the single components, while skatole attracted as many individuals as the small blend of four components including skatole (indole, skatole, 2-butanone, butyric acid). The large blend, a combination of the small blend as well as p-cresol and phenol exceeded the remaining baits' in attractivity multiple times, suggesting that p-cresol might be a key component for dung beetle attraction. This hypothesis is also underpinned by our dung VOC analyses, since it was detected in all investigated dung types. Yet, p-cresol may not be an efficient single component, because singularly baiting resulted in low attractiveness. The large blend did not differ in attraction regarding the number of species and individuals of all sampled beetles, compared to the three most attractive dung types (Fig.4), thus simulating an attractive blend for the most generalistic species. Similar results have been shown for houseflies (Cosse and Baker 1996) where a mixture of three VOCs (butyric acid, skatole, dimethyl trisulfide) was as attractive as pig dung (see also Zito et al. 2014). The GC-traces of the most attractive bait types, showed that

p-cresol and skatole are among the main VOCs in cow and wild boar dung (Fig. 3 C) and likewise a part of the most attractive chem baits. These findings might highlight olfactoric patterns for both, the community and some species and emphasize the usage of these two key substances in olfactory resource acquisition. Our results may thus be a first step in selecting key components necessary for dung beetles to locate their resources.

Acknowledgements

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6.6 **Supplementary Material to Chapter 6**

Table S6.1: Number of collected individuals per species and dung bait.

Species	Cow	Deer	Fox	Horse	Sheep	Wild boar
Anoplotrupes stercorosus	12	1	1	4	8	12
Trypocopris vernalis	2	2	2	0	2	2
Typhaeus typhoeus	10	1	0	2	2	0
Onthophagus coenobita	18	3	4	1	1	15
Onthophagus fracticornis	28	2	0	3	37	25
Onthophagus gibbulus	0	0	0	0	0	1
Onthophagus joannae	4	0	3	0	7	7
Onthophagus ovatus	33	46	20	5	105	182
Onthophagus similis	10	2	0	1	27	29
Aphodius ater	8	2	0	5	27	10
Aphodius coenosus	3	4	0	1	8	0
Aphodius corvinus	0	0	0	0	1	0
Aphodius depressus	62	3	0	2	8	4
Aphodius distinctus	4	0	0	7	17	10
Aphodius fimetarius	5	1	0	0	2	1
Aphodius luridus	2	0	0	0	4	0
Aphodius prodromus	845	85	22	240	546	919
Aphodius pusillus	19	0	0	0	0	0
Aphodius sphacelatus	24	2	2	14	27	46
Aphodius sticticus	26	0	0	2	1	6
Aphodius zenkeri	4	0	0	0	1	0

Table S6.2: Number of collected individuals per species and chem bait.

Species	2-Butanone	Small blend	Large blend	Butyric acid	Indole	p-Cresol	Phenol	Skatole
Anoplotrupes stercorosus	0	0	5	2	0	0	0	1
Onthophagus fracticornis	0	0	7	0	1	0	0	2
Onthophagus ovatus	0	0	3	2	0	0	0	0
Onthophagus similis	0	1	5	0	0	0	0	1
Aphodius ater	0	0	0	0	0	0	0	1
Aphodius coenosus	0	0	0	0	0	0	0	2
Aphodius distinctus	0	0	26	0	0	0	0	8
Aphodius prodromus	0	208	1336	0	12	25	1	325
Aphodius sphacelatus	1	4	15	0	1	3	1	3
Aphodius sticticus	0	0	10	0	0	1	0	0

Table S6.3: Gas-chromatographic compound identification for all dung scents. Compounds are listed as percentage.

Compound	Cow	Deer	Fox	Horse	Sheep	Wild boar
Me-butric acid[1]	-	-	-	-	-	13.4
Pentanoic acid	-	1.29	-	-	-	-
unstaturated alcohol 1	-	-	-	1.79	-	-
Citronellene	-	-	-	3.43	-	-
hexanoic acid	-	-	-	-	-	12.06
Benzaldehyde	-	1.64	3.07	-	2.75	-
Me-C7	-	-	-	2.12	-	0.76
2-Octen-1-ol	-	1.7	1.79	-	-	0.69
2-Methyl-2-hepten-6-one	-	6.24	-	5.97	4.37	-
C10	-	6.81	-	-	-	2.06
2-octanone	-	-	-	-	11.14	-
Phenol	8.74	-	26.05	-	-	-
Octanal	-	-	-	-	-	0.53
C8 alcohol	-	-	-	-	1.84	-
unstaturated alcohol 2	-	-	-	1.04	-	-
Cyclohexanemethanol[1]	0.4	-	-	-	-	-
Dipropyl disulfide	0.22	1.15	0.46	1.82	-	-
Limonene	-	2.28	-	0.66	-	0.41
unknown alcohol	-	2.26	-	-	-	-
Monoterpene 1	-	1.86	-	-	-	-
Isophorone	-	-	-	-	0.65	-
Methyl cyclohexanoate[1]	0.73	-	-	-	-	-
unknown	-	-	-	1.04	-	-
Acetophenone	-	4.49	-	-	5.89	-
Monoterpene 2	-	-	-	-	1.1	-
p-Cresol	31.68	47.28	22.68	49.96	60.64	65.2
Thymol	-	0.71	-	-	0.62	-
nonanal	-	3.19	0.59	21.12	2.28	1.42
Ethyl cyclohexanoate[1]	0.06	-	-	-	-	-
Camphor	0.08	-	-	-	-	-
unknown	-	1.09	-	-	1.67	-
p-Ethylphenol	56.92	-	-	-	-	-
C12 ketone	-	-	-	0.43	-	-
C12	-	-	-	0.92	-	-
unkown methyl alkane	-	-	-	-	0.77	-
Decanal	-	-	-	0.86	0.74	-
beta-Cyclocitral	-	0.68	-	-	0.94	-
Quinoline	-	-	3.21	-	-	-
p-Propylphenol	0.31	-	-	-	-	-
C13	-	-	-	1.29	-	-
1H-indole	0.67	-	41.77	-	-	1.3
Tetrahydroquinoline[1]	-	-	0.39	-	-	-
Tridecanal	-	-	-	1.17	-	-
C14	-	-	-	-	0.47	-
Sesquiterpene 1	-	-	-	1.25	-	-
beta-Caryophyllene	-	0.18	-	0.37	-	-

Skatole	0.19	0.51	-	-	-	2.1
Sesquiterpene 2	-	13.02	-	1.51	2.1	-
Sesquiterpene 3	-	-	-	0.14	-	-
Sesquiterpene 4	-	-	-	1.35	0.48	0.04
Sesquiterpene 5	-	1.66	-	-	0.18	-
Sesquiterpene 6	-	-	-	-	-	0.04
Sesquiterpene 7	-	-	-	0.43	0.17	-
Sesquiterpene 8	-	-	-	1.33	0.38	-
Sesquiterpene 9	-	1.96	-	-	0.82	-

[1] tentatively assigned

Chapter 7

7 General Discussion

Chapter 2 to 6 of this thesis provide the systematic assessment and processing of multiple surveys of dung beetle communities and their ecosystem services across a land-use gradient in forests and grasslands. As a result of the beetles' distribution and patterns in their use of resources, I additionally analyzed the nutritional composition and the role of volatile organic compounds in different dung types. In the upcoming chapter, I will discuss the results regarding the aims and initial questions of chapter 1, including a brief synopsis of the main findings.

(Q1) Does land use affect dung beetles and their ecosystem services?

Land use is known to substantially affect habitats, with altering effects for (insect) communities(Brown 1997, Newbold et al. 2015). This applies equally to dung beetles (Nichols et al. 2007), yet, I analyzed for the first time the effects of single components of land use and forestry along an intensity gradient for present dung beetle communities. In chapter 2, I found distinct habitat preference on a species level and a shift in the abundance for certain dung beetle taxa between forests and grasslands. Consequently, this is also reflected in the beetles' removal activity, as beetle densities in forests exceeded multiple times the beetles' abundance in grasslands. As mainly tunneling species, like Geotrupidae and *Onthophagus* account for the removal of dung within a short time, the monitored occurrence and distribution is crucial to predict activity of those species in context of anthropogenic disturbance.

Events of heavy disturbance, such as timber harvest and mowing decreased dung beetle abundance and thus the beetles' ecosystem service. On the other hand, both management indices (ForMI, Kahl and Bauhus, 2014 and LUI, Blüthgen et al., 2012) are characterized by a sum of management components, which revealed contrasting local effects: as timber harvest and mowing highlighted negative effects, the proportion of non-native tree

species facilitated an increase of dung beetle abundance, and the same was true for grazing. Hence, dung beetle activities were relatively balanced along the aggregate land-use intensity gradients. As a result, dung beetles seem to be able to compensate certain levels of gradual disturbance by abundance, mobility or seasonal occurrence. However, as the studies were conducted in regions that include heterogeneous managed landscapes, mostly covering a rather extensive use of meadows and forests, many of the broad risks for dung beetle communities due to intensive management are not covered. Major disturbances and regime shifts, particularly deforestation or conversion of forests to pastures, pesticides and veterinary treatments of livestock are likely to cause a much stronger decrease of dung beetle densities, resulting in declining removal activity (Nichols et al., 2007; Newbold et al., 2015). Still, the strong habitat preference among different genera suggests that there are other important environmental filters besides anthropogenic influence, such as climatic conditions or resource characteristics.

(Q2) How do dung beetle-resource connectivity and the complexity of this trophic network react to increasing land-use intensity?

Land use and forestry affects biodiversity, leading to a loss of species (Sala et al. 2000) and associated ecosystem services (Krebs et al. 1999, Tilman et al. 2001). Several studies, however, suggest that enhanced complexity of trophic networks (e.g. animal-resource connectivity) fosters higher stability of functions performed by those species (Dunne et al. 2002 Ecol Let, Memmott et al. 2004 Proceedings B). As I found dung beetles and their removal activity affected by agricultural management (chapter 2), I conducted a more indepth analysis for sampled dung beetle communities and single species in chapter 3. Surprisingly, significant effects of habitat management only occurred on single dung beetle species, while this detritivorous community itself proved high resilience towards anthropogenic disturbance. Some species either suffered (seven species declined by timber harvest) or took benefit (eight species increased with rising proportion of conifers) highlighting both, positive and negative effects of land use and forest management – similar to patterns I found for the beetles' removal activity. The most abundant species, providing the majority of collected individuals, however, remained neutral in their response towards land-use intensity.

The robustness for dung-beetle community networks persists along any land-use gradients in forests and grasslands. Higher network complexity (H_2) and network generalization (low H_2'), driven by a higher beetle abundance and diversity, increased the

total amount and evenness of dung removal across dung types. Additionally, their widespread occurrence in high numbers highlights a large adaptive capacity for certain dung beetle species. Different beetles showed contrasting habitat preferences as well as contrasting responses to land-use gradients, which leads to similar diversities and functional balance within their corresponding habitats. Such responses demonstrate the dynamics and compensatory mechanism of trophic networks beyond an overarching community level, measured and expressed by biodiversity alone.

(Q3) Do dung beetle – resource interactions change in specificity along the global, latitudinal gradient?

Classical niche theory predicts the coexistence of larger numbers of species with rising resource specificity (Stevens 1989 Am Nat 133). Hence, the increasing plant and animal diversity towards the equator has been suggested to facilitate enhanced species diversity with decreasing latitude (MacArthur 1972). Although these patterns are controversial, as several studies found differing or opposite effects (Schleuning et al. 2012, Morris et al. 2014, Novotny et al. 2006), an assessment of global specialization patterns on a detritivorous level is missing. Therefore, I quantified in chapter 4 the beetles' dung-resource specificity in a global approach, comprising 26 countries on a latitudinal gradient.

In this unprecedented approach, I confirmed a highly generalistic use of dung by dung beetle communities on a global latitudinal scale, at a comparable level as reported for generalized frugivores or nectar-seeking ants (Blüthgen et al. 2007, Schleuning et al. 2012). For the same dataset the beetles' diversity significantly increased towards the equator, highlighting that dung beetles corroborate known latitudinal diversity patterns. Several studies demonstrated competition across beetle species for dung resources can be severe, and different life-history strategies exist for rapid resource acquisition. Especially in tropical regions dung beetle species can become highly specialized on particular types of dung, such as sloth feces (Young 1981), or other food items, such as millipede carcasses (Schmitt et al. 2004), or, rarely, vertebrate carrion (Larsen et al. 2006, Scholtz et al. 2009). Still, I did not find any correlation of dung beetle specificity and dung beetle diversity. As dung beetles are able to use a broad range of resource, one explanation might be, in terms of interspecific competition, that the beetles are able to specialize on a certain resource, maintaining a high diversity and in return a "generalized" removal of dung.

Q4) Is the nutritional value of dung a driving force for dung type attractiveness and dung beetle preference?

Dung beetles are characterized as generalistic in their utilization of suitable resources (Hanski and Cambefort 1991). However, former studies (Whipple and Hoback 2012, Larsen et al. 2006 Coleopts Bull), including my own data (Frank et al. 2017a), demonstrated the beetles' ability to favor certain dung types. Field experiments in chapter 2 and 3 demonstrated a generalized usage of all offered dung types, but also a variable attractiveness for the total quantity of attracted beetles. Such differing attractivity can be driven by several factors e.g. the "host" animals' diet (carnivore, herbivore, and omnivore), which affects the nutritional value or volatile organic compounds emitted by dung (Gittings and Giller 1998 Ecography, Dormont et al. 2007), and consequently stimulated the nutritional analyses I conducted in chapter 5.

I showed that the nutritional composition was unrelated to the beetles' food selection, as I found C/N ratio, amino acids, fatty acids, cholesterol/sterol ratio and the composition of amino acids varied across dung types and feeding guild, but did not correspond to the number of beetles attracted. These analyses revealed that dung, even though already digested, still represents a valuable resource that grants sufficient amounts of most (essential) nutrients for insects. Hence, symbiotic bacteria may not be mandatory for a nutritional upgrading in the dung beetles' diet. Regarding C/N, protein (= amino acid content) and fatty acids (= NLFAs and free fatty acids), dung showed similar values to resources available for other terrestrial beetles, such as litter, fruits, fungi and carcasses. Conversely, the overlap in nutritional value of dung and alternative resources provides an explanation for the high abundance of dung beetles found during the fieldwork of this thesis, especially in forests. Finally, as I found no correlation of dung specific volatile blends and corresponding dung specific nutrients, the results of this chapter lead to a different context for (blends of) volatiles as attracting vector for dung beetles, uncoupled from nutritional values.

(Q5) Which roles have volatile organic compounds in dung beetle attraction?

In chapter 6 I finally focused on volatile organic compounds (VOCs) and their meaning in dung beetle attraction. Dung scents are known to comprise large numbers of different VOCs, including compounds that are common in all dung types (Dormont et al. 2007, 2010, Stavert et al. 2014). The mixtures of ubiquitous and particular specific VOCs in

dung provide potentially cues for a generalistic foraging and likewise selective generalist behavior of dung beetles, which I tested in a comparative approach of VOC baits and dung baits in the field.

VOC baits were able to attract only a subset of dung beetle species sampled with dung baits, still single components (i.e. skatole) were able to attract large numbers of individuals, the large blend of VOCs (comprising all six single components), furthermore, attracted an equal amount of dung beetle individuals as the most attractive dung types. The gap in attractivity of single and mixed VOCs for the dung beetle community suggests that different species use different blends to locate the resources. Dung scent analysis of all used dung types revealed that p-cresol and skatole are among the main VOCs in cow and wild boar dung and likewise a part of the most attractive chem baits. Hence, the attractiveness of the large blend not only highlights the necessity of a certain (species specific) combination of volatile components, but also suggests especially p-cresol as (enhancing) key component for dung beetle attraction. These findings highlight olfactoric patterns on a community- and species level, and emphasize in general the use of key substances in olfactory resource acquisition for dung beetles.

Conclusion and Synthesis

The ongoing need of agricultural and forest related land use is a constant factor of anthropogenic disturbance towards insect diversity, including dung beetles (Hutton and Giller 2003). Further research within this context, therefore, provides an enhanced understanding of consequences and effects for this ecologically important group of detritivores.

Chapters 2 and 3 aimed for an in-depth analysis of management effects on dung beetles and their ecosystem services. For the particular assessments of differing management intensities in forests and grasslands, I found ambivalent results. The beetles' ecosystem service is foremost negatively affected as heavy disturbance like timber harvest and mowing events reduce the present beetle abundance and consequently the amount of removed dung. The beetle community, however, seemed to withstand a certain amount of habitat management. For both chapters it is notable that the use of an overarching management index (i.e. ForMI in forests and LUI in grasslands) was insufficient to acquire clear effects in context of differing management intensities towards dung removal and community structure. For an improved analysis, the single management components of the indices provided much more information and highlighted effects of recent management. Additionally, it becomes clear that, based on the single components, the dung beetle community is able to compensate for disturbances – either way by abundance of certain species, beneficial conditions (i.e. non-native tree species in forests and grazing in grasslands) or network complexity. Again, the analysis in chapter 3 highlighted negative trends for the beetles' abundance (up to a species level), which might already be reflected in a declining removal (see chapter 2). As the beetle-resource interaction and the resulting network complexity was found to play an important role in maintaining a robust dung beetle community against anthropogenic disturbance, I conducted in chapter 4 an analysis of dung beetle diversity and resource specificity on a global scale. Latitudinal trends in increasing or decreasing resource specificity and/or beetle diversity might reveal inherently vulnerabilities for which land-use and forestry might become an additional, weakening threat. Despite of ubiquitous specialists among dung beetles, the overall trend on a global scale revealed both, generalized and specialised dung beetle – resource specificity without any trend along latitudinal distribution. Hence, the broad use of different resources (i.e. dung types) and the resulting network complexity might be a driving effect for the coexistence of large numbers of beetle species (Finke and Snyder 2008 Science, MacKane 2002 Nature).

Chapters 2-4 highlight the dung beetles adaptability towards anthropogenic disturbance, due to their ability of being generalists and to favor certain resources, such as fungi, carcasses and fruits, at the same time (Holter and Scholtz 2007,Ecol Entomol, Whipple and Hoback 2012, Frank et al. 2017a). Highly abundant and generalistic beetle species are able to compensate for minor abundant species (portfolio effect, Tilman et al. 2006, Nature) and maintain ecosystem services, provided by the dung beetle community. Consequently, this detritivorous group of insects is (within the species-specific habitat) to a certain extent unexpectedly robust in context of habitat management, compared to other insect groups (Chisté et al 2016, Kämper et al. 2016).

The nutritional analysis in chapter 5 provided novel insights for the resource quality of formerly processed food (i.e. dung). I was able to show the sufficiency of dung as primary source of mandatory nutrients for dung beetles (Frank et al 2017b). In comparison with alternative resources, such as litter, fungi, fruits and carcasses; dung showed similar values. This highlights its nutritional value and additionally the exchangeability in terms of a short supply or a lack of dung per se. As there was no correlation between the nutritional value of tested dung types and the corresponding numbers of sampled beetles, patterns in resource specificity lead consequently to cues in olfactory sensing of dung beetles, which I investigated in chapter 6. In search of drivers for present patterns of dung beetle attractivity towards the different dung types, the analysis of volatile organic compounds (VOCs) revealed ubiquitous (key) components in dung and the importance of volatile blends in dung beetle attraction (Stavert et al. 2014). Still, we need to improve our knowledge, regarding the olfactory sensing on a species level and the meaning of VOCs for dung beetles.

Outlook

Chapters 2-4 of this thesis revealed the high value of multiple surveys (intensive surveys) and wide ranged samplings (comprehensive survey) for an indepth community analyses and corresponding ecosystem services. Especially for gradient driven effects, a large number of samplings (i.e. 25-50 sampling sites per habitat and management gradient) provide the opportunity to assess slight changes and thus to predict possible consequences for the corresponding test subjects. Hence, an ongoing monitoring of dung beetles as biological indicators within this context, likewise on a local and global basis, could improve the assessment and understanding of land-use driven effects for detritivores.

The distinct habitat specificity of several dung beetle species proved to be a recurring pattern throughout the surveys. Still, decisive drivers for this species-specific habitat preference are missing. The analysis of cuticular hydrocarbons (CHCs) might reveal a temperature-driven distribution (i.e. protection against water loss), subsequently to several insect species (Kühsel et al. 2017, Brückner et al. 2017 PLOS ONE). Additionally, the amount of omnipresent resources in forests (e.g. litter and fungi) might be a driving force for larger beetle species to choose this habitat and maintain a highly abundant community, thus able to switch in resources. Experimental setups aiming for a comparing approach of CHCs and regarding the development of dung beetles and their larvae on differing resources (dung versus alternative resources) might be useful to gain a better understanding of the beetles' habitat specificity.

Chapter 5 and 6 revealed there are still only scarce information whether dung beetles use individual scents or multi-compound blends to locate and discriminate among preferred dung resources (Stavert et al. 2014). Consequently, the volatile composition of dung scent profiles analyzed within this study could provide suitable components for a broad setup of olfactory experiments regarding the dung beetles' sensing. As a first step, electrophysiological experiments, such as gas-chromatographic electro-antennographic detection (GC-EAD) could be a supporting tool for the identification of stimuli triggering components. Finally, olfactometer-based experiments for the identification of attractants or repellents and field samplings with multiple blends of volatiles are necessary, to provide supporting information for single species and on a community level.

Chapter 8

8 Appendix

8.1 Contributions to the chapters of this thesis

Chapter 2

Land use affects dung beetle communities and their ecosystem service in forests and grasslands

Kevin Frank, Marietta Hülsmann, Thorsten Assmann, Thomas Schmitt and Nico Blüthgen

K.F. and N.B. conceived the initial idea, conducted the design of the study and drafted the manuscript; K.F. carried out the fieldwork, dung sampling, identified species and conducted the formal analysis; M.H. identified species and commented on the manuscript; T.A. and T.S. participated in the design of the study and commented on the manuscript; N.B. acquired funding, designed and coordinated the overall study and conducted the formal analysis of the study. All authors declare no conflict of interest and gave final approval for publication

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Chapter 3

Improved functional performance and robustness from complex trophic networks

Kevin Frank and Nico Blüthgen

K.F. and N.B. conceived the initial idea, conducted the design of the study and drafted the manuscript; K.F. carried out the fieldwork, dung sampling, identified species and participated in the formal analysis; N.B. acquired funding, designed and coordinated the overall study and conducted the formal analysis of the study. All authors declare no conflict of interest and gave final approval for publication

Chapter 4

Global dung webs: high trophic generalism of dung beetles along the latitudinal diversity gradient

Kevin Frank, Frank-Thorsten Krell, Eleanor M. Slade, Elizabeth H. Raine, Li Yuen Chiew, Thomas Schmitt, Charles S. Vairappan, Phillippe Walter and Nico Blüthgen

K.F. and N.B. conceived the initial idea, conducted the design and formal analyses of the study and drafted the manuscript; K.F., T.S., F.T.K., P.W., E.M.S., E.R., C.S.V. and L.Y.C. carried out the field and dung sampling, identified species and/or provided unpublished datasets; T.S., F.T.K., P.W., E.S. and E.R. commented on the manuscript; N.B. acquired funding, designed and coordinated the overall study. All authors declare no conflict of interest and gave final approval for publication.

This chapter is submitted to *Ecology Letters* and currently under revision.

Chapter 5

Nutrient quality of vertebrate dung as a diet for dung beetles

Kevin Frank, Adrian Brückner, Andrea Hilpert, Michael Heethoff and Nico Blüthgen

K.F. conceived the initial idea, carried out the field and dung sampling, identified species, assisted the chemical analyses, participated in the design and formal analyses of the study and drafted the manuscript; A.B. conceived the initial idea, performed chemical analyses, carried out the statistical analyses and drafted the manuscript; A.H. collected dung and performed amino acid analyses; M.H. designed chemical analyses and discussed results; N.B. acquired funding, designed and coordinated the overall study, and drafted the manuscript. All authors declare no conflict of interest and gave final approval for publication.

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Chapter 6

In search for cues: dung beetle attraction and the significance of volatile composition in dung

Kevin Frank, Adrian Brückner, Nico Blüthgen and Thomas Schmitt

K.F. conceived the initial idea, carried out the field and dung sampling, identified species, assisted the chemical analyses, carried out the design and formal analyses of the study and drafted the manuscript; A.B. performed chemical analyses, participated in the statistical analyses and commented manuscript; N.B. acquired funding, designed and coordinated the overall study, and commented the manuscript; T.S. designed and coordinated the overall study and drafted the manuscript. All authors declare no conflict of interest and gave final approval for publication

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Ich erkläre hiermit ehrenwörtlich, dass ich die vorliegende Arbeit entsprechend den Regeln guter wissenschaftlicher Praxis selbstständig und ohne unzulässige Hilfe Dritter angefertigt habe.

Sämtliche aus fremden Quellen direkt oder indirekt übernommenen Gedanken sowie sämtliche von Anderen direkt oder indirekt übernommenen Daten, Techniken und Materialien sind als solche kenntlich gemacht. Die Arbeit wurde bisher bei keiner anderen Hochschule zu Prüfungszwecken eingereicht.

Darmstadt, den 14.03.2018

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Kevin Frank

8.6 **Curriculum Vitae**

Dipl.-Biol. Kevin Frank

Date of Birth: 27.02.1986

Place of Birth: Frankfurt a.M, Germany

Nationality: German

Higher Education and Work Experience

05/2014 – present	**PhD** at Technische Universität Darmstadt, Germany; group of Prof. Blüthgen; Thesis: "Land-use Responses of Dung Beetle Communities and their Ecosystem Services".
04/2013 – 01/2014	**Research assistant** (workgroup "Ecological Networks" of Prof. Blüthgen, Dept. of Biology, TU Darmstadt, Germany)
10/2012 – 12/2012	Student assistant (Dept. of Biology in collaboration with Dept. of Mechanical Engineering, TU Darmstadt, Germany) - assistance for an interdisciplinary project competition "emb/KIVA – Einführung in den Maschinenbau" (Mechanical Engineering, Politics, Biology, Philosophy)

Education

03/2013	**Diploma** in Biology (final grade: 1.7) Technische Universität Darmstadt, Germany; Thesis: „Effects of Temperature on Herbivory and Respiration of Orthoptera"
10/2006 - 03/2013	Student of biology at TU Darmstadt, Germany; main subjects: Ecology, Systematic Zoology, Plant Physiology
07/2002 - 05/2005	Heinrich-von-Gagern-Gymnasium, Frankfurt, Germany; Graduation: Abitur (2.5)
08/1992 - 07/2002	Freie Christliche Schule Frankfurt, Germany; primary school and secondary high school (Gymnasium)

National service

10/2005 - 06/2006	Basic Military Service (Army Lead Command of the German Armed Forces, Koblenz, Germany)

Additional Skills

Office Tools: Microsoft Office Word, Excel, Power Point

Graphic tool: Inkscape, (advanced)

Programming Tools: R-Studio (advanced), Past3 (basic)

Programming Languages: R (advanced)

Languages: German (first language), English (advanced, spoken and written)

Extracurricular activities

2003 - present	side job during high school and studies in a museum (Bibelhaus Erlebnismuseum); responsible for sales, consulting with customers, support for group management, security and maintenance
2001/2002	voluntary activities at the community of Dreeich/Götzenhain (confirmation work)

List of Publikations and Contributions

Publications

2018

Frank K., Brückner A., Blüthgen N. and Schmitt, T. (Chemoecology, in review) In search for cues: dung beetle attraction and the significance of volatile composition in dung.

Frank K., Krell F.T., Walter P., Slade E.M., Raine E.H., Chiew L.Y., Vairappan C.S., Schmitt T. and Blüthgen N. (Ecology Letters, in review) Global dung webs: high trophic generalism of dung beetles along the latitudinal diversity gradient.

2017

Frank K., Brückner A., Hilpert A., Heethoff M. and Blüthgen N. (2017b) Nutrient quality of vertebrate dung as a diet for dung beetles. Scientific Reports 7:12141

Frank, K., M. Hülsmann, T. Assmann, T. Schmitt, and N. Blüthgen (2017a) Land use affects dung beetle communities and their ecosystem services in forests and grasslands. Agriculture, Ecosystems and Environment 243:114-122

2015

Mangels J., Blüthgen N., **Frank K.**, Grassein F., Hilpert A., Mody K. (2015) Tree Species Composition and Harvest Intensity Affect Herbivore Density and Leaf Damage on Beech, Fagus sylvatica, in Different Landscape Contexts. PLoS ONE, 10(5): e0126140.

Conference contributions

46th Annual Meeting of the Ecological Society of Germany, Austria and Switzerland in Marburg,Germany, 2016 - conference talk: "Tiny engineers face modern land use: dung beetle communities, their ecosystem services and responses to management"

11th, 12th & 13th Annual Assembly of the Biodiversity Exploratories in Wernigerode, Germany, 2014-2016 - conference talk and poster